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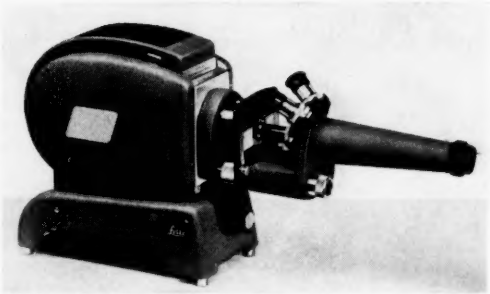

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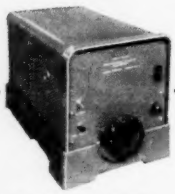

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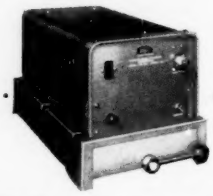

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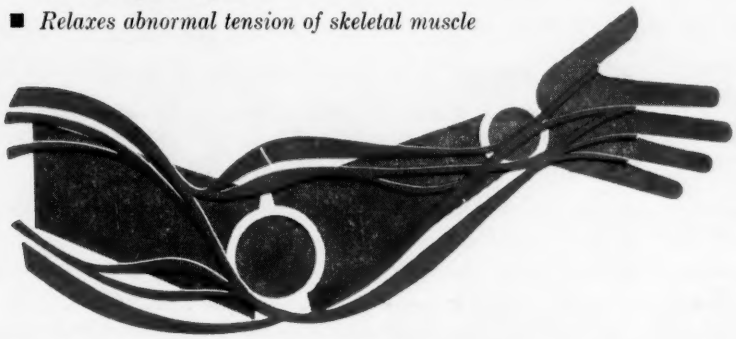

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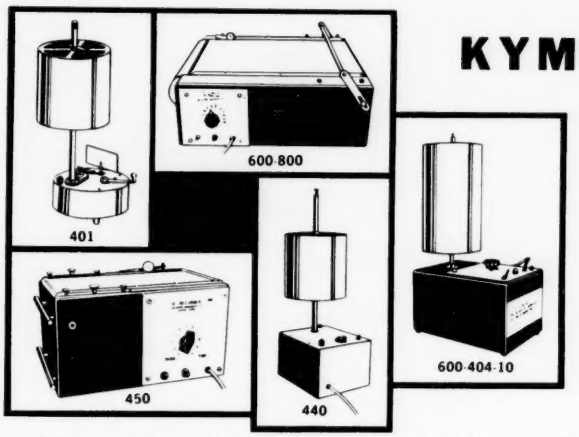

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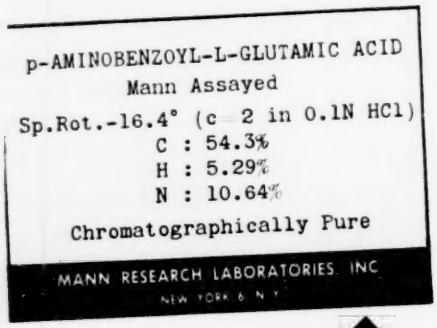

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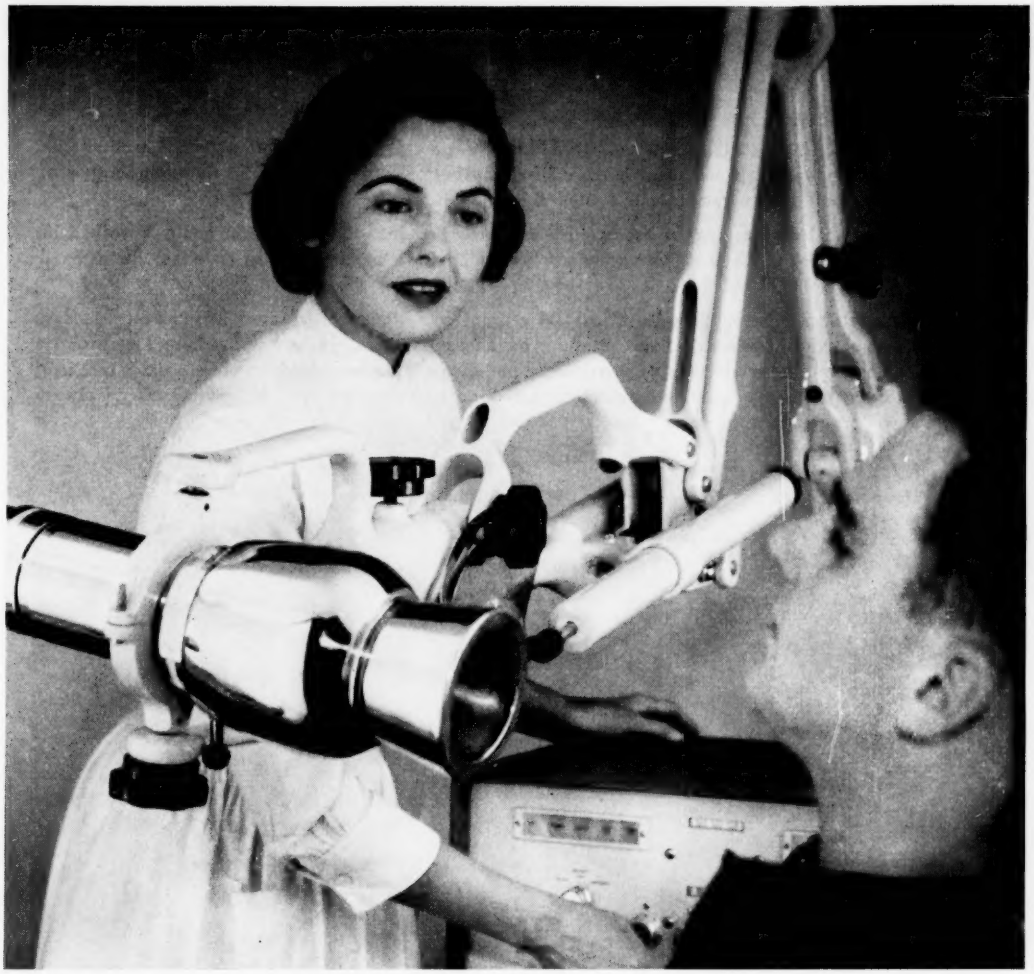

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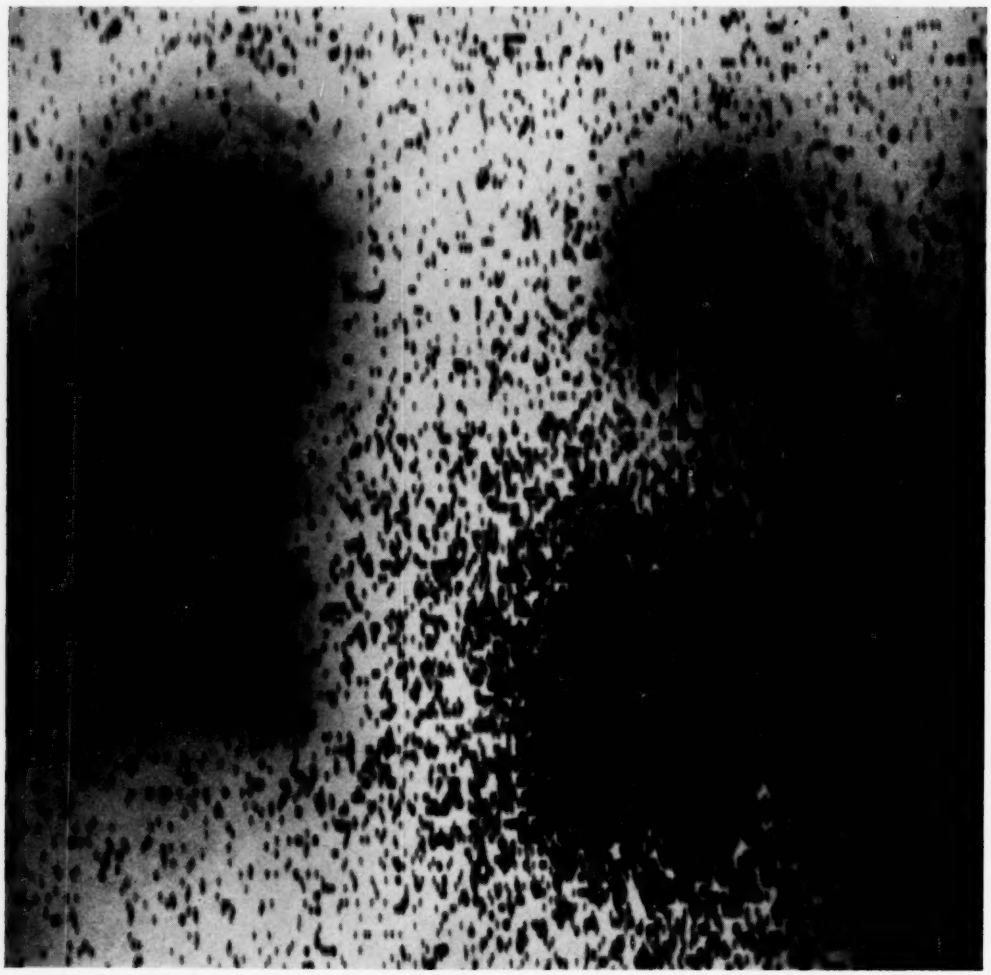

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A BIOCHEMICAL STUDY OF CERTAIN SKELETAL MUSCLE CONSTITUENTS IN HUMAN PROGRESSIVE MUSCULAR DYSTROPHY *

By PAUL J. VIGNOS, Jr. and M. LEFKOWITZ

(From the Department of Medicine, Western Reserve University School of Medicine, Cleveland, Ohio)

(Submitted for publication June 8, 1956; accepted February 5, 1959)

Many biochemical studies on skeletal muscle have been reported in experimental muscular dystrophy of animals. The effect of vitamin E deficiency on muscle metabolism has been most intensively studied. The disease of animals which most closely resembles human muscular dystrophy by genetic and histologic criteria is the hereditary primary myopathy of the mouse (1).

This mutant muscle disease of the mouse provides a useful tool for research. However, the metabolic and etiologic correspondence with human muscular dystrophy is not yet established. It would appear, therefore, that it might be worthwhile to study muscular dystrophy in man by chemical analysis of muscle obtained at biopsy from patients with this disease.

This report describes some biochemical studies on the skeletal muscles of patients with progressive muscular dystrophy. This disease is of particular interest because it is the most frequently occurring primary myopathy and may be regarded as the prototype of primary degenerative muscle disease. Skeletal muscle secondarily atrophic due to neurogenic disease of the lower motor neuron has been studied to determine whether biochemical changes in dystrophic muscle are due to nonspecific effects of muscle wasting. Diagnosis in all patients was based on combined clinical and histologic findings. Clinically, all the dystrophy patients were ambulatory and in the early stages of the disease by functional classification and extent of muscle involvement. It was hoped that any characteristic biochemical alterations might be better appreciated in the muscles of early disease rather than in the replaced and scarred tissue of advanced disease. In the latter case, similar end stage changes may be seen in any type of chronic myopathy.

* This work has been supported by a grant from the Muscular Dystrophy Association of America, Inc.

METHODS

The study was done on skeletal muscle obtained by surgical biopsy. Muscle was excised under general anesthesia in both patients and controls. One type of general anesthesia, cyclopropane, was used in all patients in order to reduce one potential source of experimental variation. Normal control muscle samples were secured from individuals with no clinical evidence of muscle weakness. The muscle was normal by histologic examination. This group was composed of seven females and three males varying in age between 2 and 47, with a median age of 30. A second group of controls had muscle disease secondary to neurogenic causes. Muscular dystrophy patients studied were ambulatory for the purpose of diminishing the factor of nonspecific disuse atrophy.

There is no universal agreement on classification in progressive muscular dystrophy. Indeed the classification of muscular dystrophy has been the source of dispute for many years (2). The clinical picture, in all 17 of the muscular dystrophy cases in this study, corresponds best with the classification of so-called "childhood type" of dystrophy described by Tyler and Stephens (3).

On the basis of the experimental biochemical data, the 17 subjects with childhood type muscular dystrophy are divided into two groups. These two groups appeared to have different metabolic patterns on the basis of the biochemical studies of muscle. The separation of the dystrophy cases into two categories has been done for the purpose of better handling of the experimental results. It is not the intention of this paper to offer still another classification of dystrophy. The clinical differences between the two groups of muscular dystrophy patients in this paper were in the age at onset of muscle weakness and the age at time of muscle biopsy. The 17 cases of muscular dystrophy were divided by these age criteria into two arbitrary groups. Those under the age of 20, at time of surgical muscle biopsy, are termed juvenile dystrophy, while those over 20 years of age at this time are called adult dystrophy. These two patient groups are further differentiated by the age when muscle weakness was first noted. The group termed juvenile dystrophy had muscle weakness beginning before the age of six years, while the so-called adult dystrophy patients' symptoms began after the age of six.

Symptoms of muscle weakness in both groups were first noted in activities involving the muscles of the pelvic girdle. No patient had the first symptoms referrable to

the muscles of the pectoral girdle. It is recognized that the history of initial muscle involvement may be misleading, but the pattern of muscle weakness in no case indicated a disproportionate early or severe involvement of the pectoral girdle or facial muscles, as is seen in the facioscapulohumeral type of dystrophy.

The pattern of muscle involvement, joint contractures and family history were similar in the two groups of muscular dystrophy. Contractures seem to be largely secondary to muscle imbalance and were present in all cases (4). There is only an occasional patient with a family history of muscle weakness. All cases showed progression of muscle weakness and there was no plateau in any patient as may be seen in polymyositis.

The pattern of individual muscle weakness was determined by manual muscle tests. All biopsied muscles used in this study were examined histologically to rule out causes of weakness other than these diagnosed on clinical grounds.

No method of grading muscle capacity in muscular dystrophy is completely satisfactory. However, since the pattern of muscle weakness in dystrophy is genetically determined, a functional classification gives worthwhile assistance in overall evaluation of the patients. This type of classification, although helpful, is less satisfactory in grading neurogenic myopathy due to the greater diversity of disease patterns. Patients are rated on a 10 point scale of functional ability based on performance of ambulation and elevation activities and on performance of activities of daily living. This is modified from the eight point scale of Swinyard, Deaver and Greenspan (5). The following criteria are used in functional classification.

1. Walks and climbs stairs without assistance.
2. Walks and climbs stairs with aid of railing.
3. Walks and climbs stairs slowly with aid of railing (over 25 seconds for eight standard steps).
4. Walks but cannot climb stairs.
5. Walks unassisted but cannot climb stairs or get out of chair.
6. Walks only with assistance or with braces.
7. In wheel chair; sits erect; can roll chair and perform bed and wheel chair activities without assistance.
8. In wheel chair; sits erect; unable to perform bed and chair activities without assistance.
9. In wheel chair; sits erect only with support; able to do only minimal activities of daily living.
10. In bed; can do no activities of daily living without assistance.

A second clinical method of assessing muscle strength was based on standard methods of manual muscle testing. To eliminate individual grading variations among different therapists, the same physical therapist did all the muscle testing in this report. Each muscle or muscle group was graded on a quantitative scale ranging from "zero" (no contraction) to "normal." This grade was then transposed to a numerical scale with zero as the lowest (no contraction) and 10 as the highest (normal) values. In order to arrive at a figure that would indicate the relative mass as well as the strength of the muscles, the numerical value was then multiplied by a factor rating ranging from one to four. The tibialis anterior muscle was selected as the muscle representing the standard of one. This factor rating is considered to remain relatively constant even with generalized atrophy. The above method was adapted from the work done to standardize muscle testing and evaluate function of the early poliomyelitis patient in relation to gamma globulin and polio vaccine trials (6). Although a complete and comprehensive muscle evaluation was done by the physical therapist, for the purposes of this study, the quantitative comparison was limited to 22 major muscles of the trunk and extremities which were considered to be a representative sampling of the muscle involvement. On this basis, a total score of 700 is the maximum that could be achieved by an individual who was rated "normal" for each muscle tested.

Muscle biopsies were taken from the rectus abdominis muscle in all controls and dystrophy patients. In three neurogenic atrophy patients this site was not considered suitable because of clinical considerations. This muscle was selected since it is involved early and uniformly in the course of childhood type muscular dystrophy and is easily obtainable from normal controls at laparotomy (3). All determinations were done in duplicate. Enzyme assays were done in duplicate and at two enzyme concentrations as a check on the linearity of the assay. Muscle specimens were wrapped in parafilm immediately after excision to prevent change in water content and immediately chilled in ice. Preparation of the muscle sample for analysis was begun within 10 minutes after removal of muscle at operation. The muscle was minced into a homogenous mash in the cold room at 4° C. The muscle mash was divided into samples of appropriate size for the determinations and wrapped in parafilm to form an air-tight package. Each sample was rapidly weighed on a Roller-Smith Torsion Balance.

Myosin extraction was begun immediately after weighing. Myosin was extracted according to the Hasselbach and Schneider (7) modification of the classic myosin extraction technique. Extraction was done in the cold (4° C.) using two periods of one hour each. Collagen was determined by the method of Lowry, Gilligan and Katersky (8). Nitrogen determinations were done by a semimicro-Kjeldahl method. Water content was determined after drying for 48 hours at 105°. Fat analysis was carried out on muscle mash samples by extraction with a 2 to 1 chloroform to methanol (v/v) mixture according to the method of Folch and associates (9, 10).

In a comparative study of diseased and normal muscle, a parenchymal reference base must be used (11). The use of an adequate tissue reference base enables the investigator to determine net quantities of tissue constituents without the error introduced by varying quantities of nonfunctional fibrous tissue, extracellular water and fat. The tissue reference base used in this study has been noncollagenous nitrogen (12).

Muscle was prepared for enzyme assays in a Potter

Elvehjem homogenizer at 4° C. The homogenizing fluid was 0.12 M KCL and 0.00016 M $KHCO_3$ at pH 7.0. A 1 to 10 homogenate was prepared. This homogenate was used for the succinic dehydrogenase assay performed by the method of Schneider and Potter (13). Creatine kinase activity was determined on the supernatant obtained by centrifugation of the original homogenate at 4° at 6,500 × G. The term creatine kinase is used to describe the enzyme which phosphorylates creatine in the presence of adenosin triphosphate (ATP) to form creatine phosphate. The reaction mixture previously described (14) has been used with addition of fluoride to inhibit residual ATP-ase activity. The creatine phosphate formed by action of the enzyme creatine kinase can be determined as "apparent orthophosphate" since, as is well known, creatine phosphate is very labile at acid pH and is completely hydrolyzed to orthophosphate and creatine during the course of procedure used for phosphorus determination. Any orthophosphate not derived from creatine phosphate is corrected for by use of control determinations having the complete reaction mixture except for creatine. Calcium activated adenosine triphosphatase activity was determined on an aqueous dilution of the original homogenate according to the method of Dubois and Potter (15).

Overall glycolysis was tested in the homogenate using a modification of the system described by LePage (16). The complete system contained phosphate buffer, 0.03 M; nicotinamide, 0.0038; pyruvate, 0.002. The incubation time was 15 minutes at 37°. The results are expressed as μg. of lactic acid formed per ml. of enzyme preparation.

TABLE I

Clinical data in disease groups studied

Subject	Sex	Age	Age at onset of disease	Functional grade	Total muscle score
		years			
A. Muscular dystrophy patients under 20 years of age					
1	M	5	1	1	607
2	M	6	3	2	537
3	M	6	3	1	506
4	M	5	4	2	472
5	M	7	3	1	426
6	M	6	4	2	383
7	M	5	3	2	342
8	M	9	4	6	338
9	M	6	2	3	336
10	M	16	3	3	316
11	M	8	5	4	245
12	M	10	4	4	175
B. Muscular dystrophy in adults					
13	M	23	7	1	489
14	M	31	19	1	
15	M	22	18	1	642
16	M	25	16	2	418
17	F	26	12	2	475
18	F	32	16	2	430
C. Neurogenic atrophy					
1	M	6	4	1	522
2	F	5	1		
3	F	3	3	2	332
4	M	2	1	6	252
5	F	½	1		
6	M	9	1	6	268
7	M	50	47	1	603
8	M	7	3	8	255

RESULTS

Clinical data on patients with muscular dystrophy and neurogenic atrophy are shown in Table I. Data in the table are arranged, in general, according to the severity of the disease at time of biopsy. The three patient groups show a similar total muscle mass.

The degeneration of muscle in progressive myopathy is associated with the appearance of connective tissue and fat cells. At histologic examination, the muscle of early dystrophy is said to show more adipose tissue than does the muscle affected by neural atrophy (17). However, no quantitative studies of the fat content of human dystrophic muscle have been performed. The muscle fat, expressed as per cent of wet weight, was increased slightly in the muscles of patients with muscular dystrophy over its content in normal control muscle (Table II). However, the increase in muscle lipid was not statistically significant. The muscle of patients with neurogenic atrophy had a similar minimal nonsignificant increase in fat content.

Fibrosis with consequent replacement of muscle by connective tissue is another result of muscle degeneration. Collagen nitrogen, expressed as per cent of total nitrogen, was significantly elevated in both juvenile dystrophy and neurogenic atrophy compared with normal controls. Three of the adult dystrophics had a definite increase in muscle collagen and there was an overall increase in this group at the 0.05 level of significance. The increase in collagen content in the juvenile dystrophic and neurogenic atrophy patients represents a proportionately greater increase in connective tissue than in fat. There was a fair correlation between the collagen content of diseased muscle and the clinical status measured by either total muscle strength or functional classification.

The water content in normal control muscle agrees with the values of Barnes, Gordon and Cope (18). Water content of muscle in all patient groups was always slightly less than average water content of muscle from normal control subjects (Table II). Juvenile dystrophy showed

TABLE II

Muscle fat, collagen and water content

Subject	Fat as % of wet weight			Collagen collagen N as % of total N			Water as % of wet weight		
	Normal controls	Juvenile dystrophy	Neurog. atrophy	Normal controls	Juvenile dystrophy	Neurog. atrophy	Normal controls	Juvenile dystrophy	Neurog. atrophy
no.									
1	1.42		2.80	5.00	32.7	9.2	80.1	66.4	78.4
2	2.06	1.40	3.10	4.25	19.4	21.8	80.7	77.4	83.8
3	2.90	2.02		5.63		15.1	79.1		79.7
4	2.51	1.75	1.76	7.41	26.3	14.8	83.1	78.0	80.9
5	1.57	6.32	1.51	10.19	22.7	31.8	83.1	77.7	78.5
6	6.19	2.34	5.41	5.95	27.5	25.0	77.3	78.4	71.4
7	0.62	4.55	5.51	4.88	31.5	32.0	80.5	76.3	79.3
8	1.25	5.13	7.33	4.60	36.8	17.5	81.9	79.5	73.1
9	0.64	2.01		5.03	28.4		81.6	77.9	
10	1.80	5.70		4.86	14.4		84.3	77.0	
11		5.14			25.6			77.2	
12		4.21			24.7			77.9	
Mean	2.10	3.68	3.52	5.78	26.4	20.9	81.2	76.7	78.1
S.D.	1.61	1.83	2.19	1.79	6.24	8.27	2.1	3.51	4.04
p		>0.50	>0.50		<0.01	<0.01		<0.01	<0.05
		Adult dystrophy			Adult dystrophy			Adult dystrophy	
13		1.80			6.4			80.2	
14		2.35			4.7			82.2	
15		0.69			8.9			83.0	
16		2.20			16.6			80.3	
17		10.35			38.6			76.9	
18		2.82			13.7			78.7	
Mean		3.36			14.80			80.20	
S.D.		3.50			12.40			2.20	
p		<0.40			<0.05			<0.40	

the greatest reduction in muscle water content. The difference from normal control muscle water is significant statistically. Neurogenic atrophy showed a lesser reduction in muscle water content while adult dystrophy had essentially normal muscle water. The reduced water content in atrophic human muscle contrasts with the increase in muscle water reported in experimental muscular atrophy of animals (19).

An enzyme of particular interest in muscle is creatine kinase. Muscle is unique among tissues in its high content of creatine phosphate; hence, any change in creatine kinase would be pertinent. The mean enzyme content of all disease groups was decreased. The decrease in juvenile dystrophic and neurogenic muscle was of greater statistical significance (Table III).

In view of the importance of ATP in muscle metabolism, adenosine triphosphatase activity was examined even though its role in the complex reactions of intact muscle may be more involved than a direct hydrolytic cleavage of ATP. Adenosine triphosphatase activity was essentially normal in adult dystrophy but showed a reduction in juvenile dystrophy and neurogenic atrophy (Table III).

Succinic dehydrogenase (Table III) was measured as a representative enzyme of aerobic respiration. There was no change in enzyme activity in dystrophy. Secondary neurogenic atrophy showed a definite decrement but the small sample makes this result of questionable significance.

The most striking enzymatic alteration was the decrease in overall glycolytic activity in juvenile dystrophy. The reduction to approximately one-third control activity is a relatively greater reduction per unit of parenchymal tissue than that exhibited by the other enzymes studied. This reduction in glycolysis is in contrast to adult dystrophy, where the muscle shows a slight reduction based on wet weight, but does not vary significantly when the activity is based on noncollagenous nitrogen

TABLE III

Enzyme activity of skeletal muscle

Subject	Creatine kinase			Adenosine triphosphatase			Succinic dehydrogenase		
	Normal controls	Juvenile dystrophy	Neurog. atrophy	Normal controls	Juvenile dystrophy	Neurog. atrophy	Normal controls	Juvenile dystrophy	Neurog. atrophy
no.	(Activity per mg. of noncollagenous nitrogen)								
1	93	33	64	384	291	114			
2	75	68	73	225	217	357	128	170	35
3	131		64	227		285	277		51
4	119	37	48	326	207	99	228		
5	114	55		322	240		169		
6	100	76	48	423	204	97	216	169	145
7	94	60	94	265	294	112	262	245	210
8	80	66	36	304	129	110	281	338	
9	62	51			344		241	42	
10	98	78		322	168		169	215	
11		107			202			242	
12		51			170			189	
Mean	97	62	61	311	224	168	219	201	110
S.D.	20.98	20.71	19.20	66.1	63.5	36.1	55.72	71.92	82.34
p		<0.01	<0.01		<0.01	<0.01		>0.50	<0.02
		Adult dystrophy			Adult dystrophy			Adult dystrophy	
13		106			425			37	
14		61			465			444	
15		68			290			285	
16		42			114				
17		92			197			37	
18		81			96				
Mean		75			264			201	
S.D.		22.8			156			199.7	
p		>0.05			>0.50			>0.50	

reference base. The decreased glycolysis in juvenile dystrophy is not specific since a similar proportional decrease was found in neurogenic atrophy.

One theory concerning the cause of muscular dystrophy implicates the contractile protein of muscle. Myosin, as the principal contractile protein component of the myofibril, was determined in an attempt to test this concept. Myosin determinations in normal control muscle show good agreement and the mean average of control values was 30.7 mg. of myosin-N per 100 mg. of noncollagenous nitrogen (Table IV). Both types of dystrophic muscle show a decrease in myosin content whether based on weight of wet muscle or on a parenchymal reference base of noncollagenous nitrogen. The muscle of adult dystrophy shows a greater decrease in myosin than juvenile dystrophy and the difference in myosin content based on noncollagenous nitrogen between adult and juvenile dystrophy is significant. Myosin content was not decreased in the neurogenic atrophy group.

DISCUSSION

The cause of human muscular dystrophy is unknown. Many theories for its etiology have been advanced. One recent concept, previously referred to, suggests that the contractile mechanism may be defective because of a failure in synthesis of proper quantities or qualities of myosin (20). A second suggestion is that the energy supply to the contractile fibril mechanism is faulty (21). In the latter case, the metabolic error may be attributed to some step or steps in the generation of high energy phosphate bonds which serve as the immediate source of energy for the muscle contraction of the myofibril.

In a consideration of the contractile function of muscle, the principal protein of the myofibril is myosin. It has been suggested that its concentration should bear an important quantitative relationship to the total mass of contractile pa-

TABLE IV

Glycolytic activity and myosin content of voluntary muscle

Subject	Myosin: Mg. myosin per 100 mg. wet muscle			Myosin: Mg. myosin N per 100 mg. noncollagen N			Glycolysis: Activity units per 100 mg. wet muscle			Glycolysis: Activity units per 100 mg. noncollagen N		
	Normal controls	Juvenile dystroph.	Neurog. atrophy	Normal controls	Juvenile dystroph.	Neurog. atrophy	Normal controls	Juvenile dystroph.	Neurog. atrophy	Normal controls	Juvenile dystroph.	Neurog. atrophy
no.												
1	4.89	2.24	4.26	31.5	18.6	33 0	6,230	670	983	2,410	335	459
2	3 92	4.01	3.60	28.3	28.4	32.4	7,130	2,040	1,870	3,100	867	1,010
3	4.30	3.16	3.97	33.9	32.2	32.1	3,375		2,310	1,600		1,130
4	2.95	3.28	4.29	25.3	26.6	31.3	3,730	1,363	1,300	1,930	665	570
5	4.58	3.14	2.70	32.8	25.3	27.2	4,050	1,630		1,750	790	
6	3.40	2.76	3.36	30.9	25.0	26.4	3,100	1,300	670	1,695	710	318
7	4.74	2.44	2.44	32.8	21.1	16.4	5,700	1,775	1,572	2,380	924	1,060
8	4.09	2 51	2 24	31 8	30.7	19 1	5,730	1,300	960	2,680	955	492
9	4 61	3.25		27.3	28.2		5,100	1,575		1,810	824	
10	4.47	3.68		32.2	30.5		6,100	1,350		2,640	675	
11		3.08			28.6			1,725			968	
12		2.91			24.6			1,560			976	
Mean	4.20	3.04	3.38	30.7	26.7	27.2	5.024	1,481	1,380	2,200	790	719
S.D.	0.62	0.53	0.81	2.8	3.99	6.40	1,300	356	575	511	190	335
p		<0.01	<0.05		<0.02	>0.20		<0.01	<0.01		<0.01	<0.01
	Adult dystrophy			Adult dystrophy			Adult dystrophy			Adult dystrophy		
13	3.96			26.4			7,150			2,900		
14	3.21			27.2			4,100			2,090		
15	3.92			19.6			3,980			1,900		
16	3.49			15.6			5,900			2,650		
17	0.81			11.3			720			605		
18	2.48			13.1			4,225			2,218		
Mean	2.97			18.86			4,350			2,060		
S.D.	1.10			6.60			2,170			802		
p	<0.02			<0.01			>0.40			>0.50		

renchymal tissue (11). If the metabolic fault in muscular dystrophy is in the contractile element of the myofibril, a disproportionate quantitative decrease in myosin content in comparison to other parenchymal muscle constituents, such as the enzyme systems involved in the transfer and production of high energy phosphate, might occur. The experiments indicate that myosin is reduced. However, the average reduction in myosin content is markedly less than that of glycolytic activity and slightly less than the reduction in creatine kinase and ATP-ase activity in the group of patients termed "juvenile dystrophy." Although the decrease in myosin content of dystrophic muscle is only moderate in degree, the possibility of a more significant qualitative alteration cannot be ruled out by these experiments.

In human muscular dystrophy, the only previously reported metabolic alterations in enzyme activity have been decreases in overall glycogenolysis and in some individual enzymes of this system, including phosphorylase, phosphoglucomutase and aldolase (22, 23). The results reported in this paper confirm the absolute decrease of glycolysis in muscular dystrophy but limit this alteration to cases classified here as juvenile dystrophy. The percentile decrease in muscle glycolytic activity to one-third normal activity is in close agreement with the results of Schapira, Dreyfus, Schapira and Kruh (23). The reduction in glycolysis is considerably greater than the absolute decrease of myosin content, adenosine triphosphatase, or creatine kinase activity. The severe glycolytic alteration in juvenile dystrophics, with only moderate muscle weakness, points to the possible magnitude of the metabolic derangement of muscle in patients with advanced muscle wasting. The role of lactic acid production in energy generation by muscle in states of relative anoxia is well known. Recent experimental evidence suggests that lactate

production may also be significant in the resting state (24). The role of glycolysis in human skeletal muscle metabolism may be of greater importance than has been believed in the past.

All enzyme systems tested including glycolysis are well preserved in the adult with dystrophy. This is particularly impressive when contrasted with the loss of enzyme activity in the juvenile dystrophic. This may represent merely a quantitative difference in disease severity between the two groups. However, the disproportionate decrease in myosin in the adult dystrophic in the face of well maintained enzymatic activity suggests that there may be a different metabolic defect in the two groups.

Dreyfus, Schapira and Schapira (22) speculate whether the muscle weakness in muscular dystrophy is due to enzymatic defects in glycolysis or whether these alterations in metabolism are the sequelae of a more primary biochemical flaw. This present study confirms the decrease in glycolytic activity, but also indicates that there is a significant reduction in myosin concentration and in nonglycolytic enzyme systems involved in high energy phosphate transfer. This indicates that the metabolic deficit in muscular dystrophy is more widespread than suggested by the work of Dreyfus and associates. The present data suggest that the biochemical alterations in dystrophy are complex and involve both myofibrillar and sarcoplasmic components. The disproportionate reduction in activity of the glycolytic system in human dystrophy suggests that further study of the individual components of this system in human dystrophy might be rewarding.

In general, similar quantitative alterations in enzyme activity are found in neurogenic atrophy and juvenile muscular dystrophy. Therefore, none of the changes are specific for the skeletal muscle weakness in muscular dystrophy. The similarity in metabolic alterations in myogenic and neurogenic muscle disease is susceptible to various alternative theories. A common enzyme or cofactor deficiency may lead to the failure of adequate lactic acid production in both types of disease or a stereotyped deficit may result from fundamentally different metabolic errors.

Another possible explanation is that as a result of disease there is an alteration in muscle membrane permeability. It has been demonstrated that the intracellular enzyme, aldolase, diffuses more rapidly from dystrophic mouse muscle than from normal muscle (25). The low content of intracellular muscle enzymes in human dystrophy could be explained by this mechanism. A similar nonspecific alteration of muscle membrane permeability could occur in neurogenic muscle atrophy. The normal serum aldolase in neurogenic disease of muscle is, however, somewhat against this theory (26).

A continuing problem in assessing the degree of skeletal muscle involvement in myopathy is the factor of tissue heterogeneity. Histologic preparations suffer from the handicap that individual microscopic sections may show widely disparate pictures. It is often difficult for the pathologist to give more than a semiquantitative evaluation of the extent of muscle disease. Quantitative chemical determination of skeletal muscle constituents, in which both the functional and replacement elements of muscle are measured, may aid in the clinical evaluation of muscle disease. These values, which are based on aliquots taken from several grams of homogenized tissue, are probably more representative of overall mean tissue composition in a given muscle than are the individual histologic sections which represent only a few milligrams of muscle tissue. It has been reported that the fat content of diseased dystrophic muscle may vary by 50 per cent in two different areas (11). Quantitative fat determinations in the group of patients reported in this paper, who have only moderate muscle involvement, vary within a relatively small range. The past reports of marked differences and overall increase in the fat content of dystrophic muscle may well reflect the long duration of the disease process (17) rather than a fundamental alteration of fat metabolism or accelerated lipid deposition in dystrophic muscle. It would appear that in early muscle disease the extent of fat deposition in muscle tissue has no diagnostic significance and, indeed, shows no significant increase over normal control muscle. It seems unwise, therefore, to interpret fat replacement of muscle as indicating a specific metabolic fat defect in muscular dystrophy.

Conversely, a significant increase in the fibrous tissue content of muscle is an early finding in ju-

venile muscular dystrophy. This would seem to be a more dependable indication of early muscle dysfunction in this disease than an increase in muscle fat.

SUMMARY

1. The content of myosin, creatine kinase, adenosine triphosphatase, succinic dehydrogenase, overall glycolysis, fat, collagen and water in the skeletal muscle of patients with muscular dystrophy has been determined. This has been compared with skeletal muscle analysis in normal controls and neurogenic atrophy.

2. The collagen content of juvenile dystrophic and neurogenic atrophy muscle was increased. The water content of muscle in these two patient groups was reduced slightly. Muscle fat was not significantly altered in any patient group.

3. In juvenile and adult dystrophy, the myosin content related to noncollagenous nitrogen was reduced.

4. Glycolysis showed the most striking decrease of the parenchymal tissue elements tested. This was reduced to one-third of normal in juvenile dystrophy. Since a similar reduction was found in neurogenic atrophy, this is not the specific metabolic derangement in dystrophy.

5. Adenosine triphosphatase and creatine kinase activity were reduced in both juvenile dystrophy and neurogenic atrophy.

6. The muscle of adult dystrophy showed no enzyme alterations although the myosin content was reduced to a greater extent than in the other two patient groups.

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CORRECTION

Footnote 1 to the article by Hoffman and Rowe, entitled "Some Factors Affecting Indicator Dilution Curves in the Presence and Absence of Valvular Incompetence," which appeared on page 138, volume 38, 1959, should read as follows: "Material supplementary to this article has been deposited as Document Number 5897 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C. A copy may be secured by citing the Document Number and by remitting $1.25 for photoprints or 35 mm. microfilm. Advance payment is required. Make checks or money orders payable to: Chief, Photoduplication Service, Library of Congress."

COMBINED ANTERIOR PITUITARY AND NEUROHYPOPHYSEAL INSUFFICIENCY. STUDIES OF BODY FLUID SPACES AND RENAL FUNCTION *

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Lesions of the neurohypophysis resulting in deficiency of antidiuretic hormone (ADH) produce the clinical syndrome of diabetes insipidus, *provided* the anterior pituitary is functionally intact. If the anterior lobe is destroyed, manifestations of diabetes insipidus may not appear (1–3). The administration of cortisone or adrenocorticotropin (ACTH) to patients with anterior pituitary insufficiency led to the recognition of coexisting neurohypophyseal failure (4–7). The presence of such combined defects is of physiological interest in that it approaches a condition in which the role of the kidney may be investigated in the human independent of hormonal control. The present study is an attempt at evaluating water metabolism and certain aspects of renal function in two patients with combined anterior pituitary and neurohypophyseal lesions.

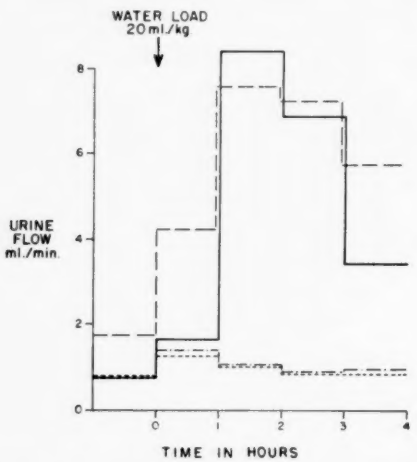

FIG. 1. CASE 1: THE RESPONSE TO ACUTE ORAL WATER LOADING

– – – –, without treatment; —·—·—, on triiodothyronine, 20 μg. three times a day; ———, after two days of cortisone, 50 mg. twice daily; and — — —, after two days of corticotropin, 40 mg. twice daily.

CASE REPORTS

Case 1. A 25 year old white male was admitted to the Middlesex Hospital for investigation of hypogonadism. Physical examination was unremarkable except for a youthful facial appearance, eunuchoidal body proportions and absent secondary sexual characteristics. The visual fields were normal. X-rays of the skull showed a beaten silver appearance with the pituitary fossa ballooned to 2 cm. in diameter. X-rays of the bones indicated a bone age of 14 years. Hemoglobin, total and differential white cell counts, blood urea, cholesterol, uric acid, alkaline phosphatase and serum electrolytes were within normal limits on repeated examination. Oral glucose tolerance and intravenous insulin sensitivity tests were also normal. An electrocardiogram showed low T waves and small QRS complexes in all leads compatible with a diagnosis of hypothyroidism. The BMR was −25 per cent (Robertson Reid Standard) but the thyroidal uptake of radioactive iodine was within the normal range. A standard water loading test (20 ml. per Kg. body weight) showed 19 per cent excretion in four hours (Figure 1). The Kepler-Robinson-Power index was 5.2. Urinary excretion of adrenal steroids was negligible but rose to high values after a standard ACTH test (8). While receiving ACTH the patient developed polydipsia and polyuria with a urine flow of over 5 L. per day. These findings suggested ADH deficiency in addition to adenohypophyseal failure. An intravenous saline infusion test supported this assumption (Figure 2).

Case 2. A nine year old white male was seen in the Harriet Lane Home of the Johns Hopkins Hospital in 1950 because of the recent onset of defective vision. The following year headaches and occasional vomiting appeared. Examination revealed a left temporal field defect. X-rays of the skull demonstrated erosion of the sella turcica.

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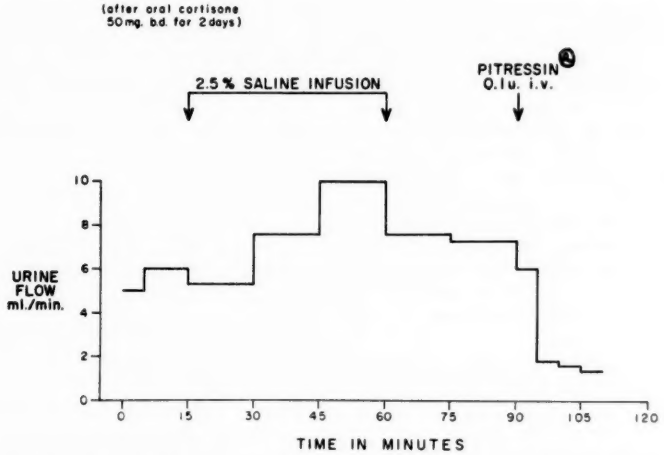

FIG. 2. CASE 1: INTRAVENOUS HYPERTONIC SALINE TEST (0.25 ML. OF 2.5 PER CENT SALINE PER KG. PER MINUTE FOR 45 MINUTES)

There was no endogenous ADH release but following the injection of Pitressin®, there was a fall in urine flow indicating normal responsiveness of the renal tubules.

In March, 1953, craniotomy was carried out with partial removal of a tumor which histologically proved to be an aberrant pinealoma. Following operation the patient received 5,000 r to the pituitary region over a period of three weeks. Within three months diabetes insipidus developed and treatment was instituted. For the next four years he remained asymptomatic while receiving Pitressin® (vasopressin), although growth lagged and puberty failed to occur. Follicle stimulating hormone remained absent from the urine and 17-ketosteroid excretion never exceeded 1.4 mg. per 24 hours. At the age of 14 years, substitution therapy with intramuscular depot testosterone was begun. Over the next 12 months physical and mental sluggishness became prominent and the protein-bound iodine fell to 1.9 μg. per 100 ml. Considerable improvement was noted following thyroid therapy.

Early in 1957 the boy noted that omission of Pitressin® caused no return of thirst or polyuria and so he stopped using it. The spontaneous disappearance of diabetes insipidus prompted re-evaluation at the age of 15.5 years. All therapy was discontinued four weeks prior to the present investigation.

Physical examination revealed slight stunting of growth, hypogonadism and sexual immaturity. There was a left temporal field defect, an unresponsive left pupil and left optic atrophy. Red and white blood counts and routine chemical determinations were normal except for a serum cholesterol of 334 mg. per 100 ml. and a protein-bound iodine of 1.9 μg. per 100 ml. Urinary 17-ketosteroids were 3.8 mg. per 24 hours and 17-hydroxycorticoids were absent from both urine and plasma. The water loading test showed a 9 per cent excretion in four hours. Following a test dose of cortisone polyuria and polydipsia returned and an intravenous saline infusion test suggested absence of ADH.

PROCEDURE

The subjects received a routine ward diet with fluids *ad lib.* Fluid intake was measured and urine output collected in 24 hour periods. Fasting daily weights were recorded at the beginning of each collection. All studies were performed following 14 hours of fasting and fluid deprivation. Information was obtained on renal function in Case 1 in the untreated state, following thyroid, and again after combined thyroid plus cortisone therapy and in Case 2 in the untreated state and on combined thyroid plus cortisone therapy at two dose levels.

Renal hemodynamics were assessed by measurements of renal plasma flow and glomerular filtration rate. After baseline values were obtained, the excretion of water and solute over a four hour observation period following oral water loading (20 ml. per Kg. body weight) were also measured. The following morning studies were made of water and solute excretion following oral urea loading (15 Gm. urea in 50 ml. of water). In Case 1, total body water and extracellular fluid volume were measured simultaneously with the glomerular filtration rate and renal plasma flow.

Following control studies, Case 1 was given thyrotropic hormone, 10 mg. daily for two days, which increased radioactive iodine uptake by the thyroid gland from 40 to 54 per cent. Triiodothyronine, 20 μg. three times a day was then substituted and after three days of this treatment, studies were repeated. Triiodothyronine was continued, oral cortisone (12.5 mg. every eight hours)

was added and after five days of this combined treatment, the studies, except for urea loading, were again repeated. In the course of the second study (on thyroid), and the third (on combined thyroid plus cortisone therapy), the acute effects of intravenous hydrocortisone-hemisuccinate (50 mg.) on fluid spaces and renal hemodynamics were also studied. Changes in inulin space were calculated by a method previously published (9). Prompt water diuresis was still impaired on combined thyroid plus cortisone therapy, with the last dose of cortisone given four hours before water loading. Observations on water and solute excretion, though not of renal hemodynamics, were therefore repeated after the dose of cortisone was increased to 100 mg. four hours before water or urea administration.

In Case 2, following control observations, substitution therapy consisting of desiccated thyroid, 120 mg. daily, and intramuscular cortisone, 5 mg. twice daily, was given. On the seventh day of therapy, the studies, except for urea loading, were repeated. The cortisone dosage was then doubled and the same studies were again made on the seventh day of this regimen.

Control subjects. Studies of the responses to oral water and urea loading, carried out under the same conditions of fasting and fluid deprivation outlined above, were obtained in a patient with untreated diabetes insipidus, a patient with uncomplicated and untreated anterior pituitary insufficiency, and a normal subject. Studies were not repeated following hormone administration nor were renal hemodynamics investigated.

Total body water was measured by the volume of distribution of antipyrine (10) and extracellular fluid volume by the distribution of inulin (11). Inulin was determined by the resorcinol-thiourea method (12). Glomerular filtration rate was taken as the clearance of inulin, and renal plasma flow as the clearance of para-aminohippuric acid (13). The factor 0.9 was used for correction for plasma water. No correction was made for renal dead space in calculating extracellular water. Urine and plasma osmolalities were determined by freezing point depression using a modified Beckman thermometer in Case 1 and a Fiske Associates Osmometer in Case 2. Osmolal and free water clearances were calculated using the formulae of Wesson and Anslow (14). Sodium and potassium were determined by flame photometry and chloride by the potentiometric method of Sanderson (15). Urea was estimated by a modification of the method of Barrett (16).

RESULTS

Case 1

Body fluid compartments (*Table I*). The relationship of total body water and extracellular fluid volume to body weight was within normal limits in the untreated patient. Thyroid decreased body weight, total body water and extracellular fluid volume. The addition of cortisone further reduced body weight and total body water but caused no alteration in extracellular fluid volume either during prolonged oral or intravenous administration.

Clearance studies (*Table I*). In the untreated patient, glomerular filtration was markedly reduced. Renal plasma flow was not measured. Thyroid raised the glomerular filtration rate; however, it was not returned to normal. The addition of cortisone returned the glomerular filtration rate and renal plasma flow to normal.

In the acute experiments, intravenous hydrocortisone increased the glomerular filtration rate when superimposed both on thyroid and on combined thyroid plus cortisone therapy. The effect on renal plasma flow was less consistent. With steroid infusion superimposed on thyroid, renal plasma flow increased appreciably. On thyroid plus cortisone administration, renal plasma flow

TABLE I

The effect of hormone administration on the body fluid compartments, renal plasma flow, glomerular filtration rate and electrolyte excretion of Case 1

	Date	Body wgt.	TBW (V_A)	ECF (V_{IN})	RPF* (C_{PAH})	GFR* (C_{IN})	Plasma Na	C_{Na}	Plasma K	C_K
		Kg.	*L.*	*L.*	*ml./min.*	*ml./min.*	*mEq./L.*	*ml./min.*	*mEq./L.*	*ml./min.*
I. Control period	6/14/56	64.8	29.0	11.2		69	140	0.460	3.6	4.78
II. On triiodothyronine, 20 μg. t.i.d.	6/22/56	63.2	27.8	10.3	470	96	147	0.659	3.8	4.61
60 min. after I.V. hydrocortisone				10.5	610	111	145	0.643	3.7	5.07
III. On triiodothyronine and 37.5 mg. cortisone	6/28/56	61.9	26.4	10.8	527	116	141	1.23	3.6	6.48
60 min. after I.V. hydrocortisone				10.9	545	126	140	1.35	3.7	7.25

* Mean of three collection periods, corrected to standard surface area of 1.73 $M.^2$.

TABLE II

The effect of therapy on renal hemodynamics, urine volumes, urine osmolalities and solute output in the two subjects with anterior and posterior pituitary insufficiency

	Medication	RPF*	GFR*	FF	Mean urine volume	Mean osmolality	Mean total solute excretion
		ml./min.	*ml./min.*		*ml./24 hrs.*	*mOsm./L.*	*mOsm./24 hrs.*
Case I	None		69		2,260 (2,100–2,400)	470 (600–440)	1,060
	Triiodothyronine, 20 μg. t.i.d.	470	96	0.202	2,270 (2,200–2,500)	425 (480–360)	970
	Triiodothyronine, 20 μg. t.i.d Cortisone, 37.5 mg. daily	527	116	0.220	4,619† S.E. ±456	278† S.E. ±21	1,250†
Case II	None	460	103	0.224	3,300 S.E. ±111	320 S.E. ±31	1,080
	Thyroid, 120 mg. q.d. I.M. cortisone, 5 mg. b.i.d.	620	121	0.196	4,760 S.E. ±156	225 S.E. ±19	1,057
	Thyroid, 120 mg. q.d. I.M. cortisone, 10 mg. b.i.d.	650	141	0.216	5,000 S.E. ±224	255 S.E. ±12	1,270

* Mean of three collection periods corrected to standard surface area of 1.73 M.².
† Excludes days when 100 mg. cortisone was given.

was higher than on thyroid alone and the response to intravenous hydrocortisone was within limits of experimental error. Intravenous hydrocortisone produced no appreciable change in sodium excretion, but enhanced potassium excretion when superimposed both on thyroid and combined thyroid plus cortisone therapy.

Twenty-four hour urine and solute excretion (Table II, Figure 3). The control 24 hour urine specimens were distinctly hypertonic. Thyroid administration reduced urine osmolality but had little effect on urine volumes. Total daily solute excretion was less. The addition of cortisone induced polyuria with the production of an approximately isotonic urine, except when the larger dosage of cortisone (100 mg.) was given. With this larger dosage, urine volumes exceeded 6 L. and urine osmolalities fell below 200 mOsm. per Kg. Cortisone increased total daily solute excretion.

Response to water and urea loading (Figure 4). The untreated patient was unable to excrete a water load promptly. Urine flow remained below 2 ml. per minute and fell progressively, as did solute excretion. Urine osmolalities remained virtually unchanged at approximately 600 mOsm. per Kg., although plasma osmolalities dropped from 315 to 290 mOsm. per Kg. Urea loading produced an increased urine flow. Urine osmolality dropped to 510 mOsm. per Kg. in the third hour but rose again thereafter. Approximately 20 per cent of the urea ingested was excreted over the four hour period. Thyroid appeared to have no significant effect on water or urea load excre-

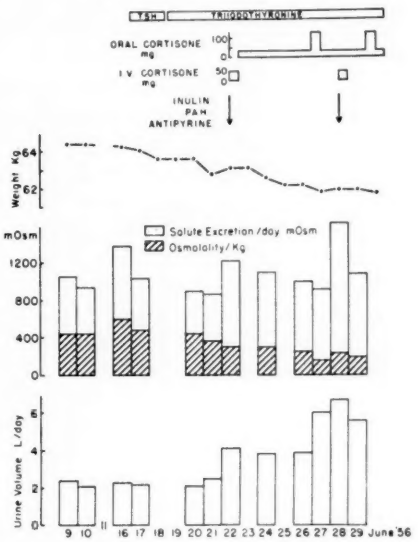

FIG. 3. CASE 1: THE EFFECT OF HORMONE THERAPY ON BODY WEIGHT, 24 HOUR URINE VOLUMES, URINE OSMOLALITIES AND TOTAL SOLUTE EXCRETION

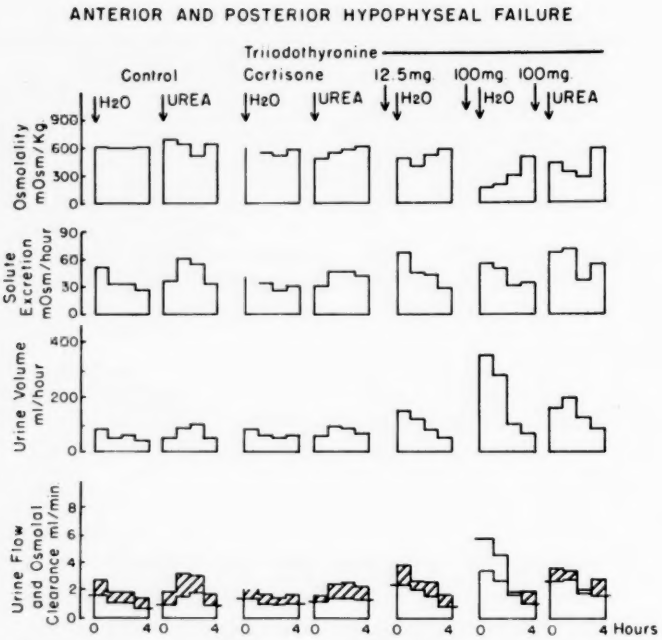

FIG. 4. CASE 1: THE RESPONSES TO ORAL WATER AND UREA LOADING

Urine volumes, osmolalities and solute excretion are shown for each of the four hourly observation periods. The bottom row shows urine flow and osmolal clearance. Where osmolal clearance exceeds urine flow the shaded area indicates the amount of solute-free water abstracted in the concentrating operation. Where urine flow exceeds osmolal clearance, the clear area above the blocks indicates free water clearance.

tion. When 12.5 mg. oral cortisone was given four hours before water ingestion, urine flow rose to 2.42 ml. per minute and solute excretion to 1.12 mOsm. per minute, respectively. Urine osmolalities, although somewhat lower, never fell below 382 mOsm. per Kg. When 100 mg. cortisone was given four hours before the test, urine osmolalities dropped to 159 mOsm. per Kg. and urine flow rose to 5.75 ml. per minute. Free water clearance was established. Cortisone had no effect on solute excretion following urea. Approximately 20 per cent of the urea ingested was excreted over four hours, as in the control study.

Case 2

The findings were similar to those in Case 1, except for quantitative differences. Clearance studies, 24 hour urine volumes and solute excretion are summarized in Table II. Although total daily solute excretion was of similar magnitude in the two patients, the 24 hour urine volumes in Case 2 were greater and urine osmolalities lower. The control glomerular filtration rate was considerably higher and approached normal. On combined thyroid plus cortisone therapy, the glomerular filtration rate and renal plasma flow rose to normal. There was again a significant increase in urine volume and a fall in urine osmolality, with the production of a hypotonic urine and overt diabetes insipidus. Total daily solute excretion rose as in Case 1 when the dose of cortisone was increased.

Control responses to water and urea loading were similar to those of Case 1. Urine osmolalities remained at approximately 500 mOsm. per Kg. Urine flow and solute excretion varied in the same direction. The response to water loading on thyroid plus cortisone is shown in Figure 5.

There was no difference in response using the larger dose of cortisone. In both instances free water clearance was established although only half the water taken was eliminated. Plasma osmolalities at the time the water load was given were 320 mOsm. and 330 mOsm. per Kg. and fell to 290 and 304 mOsm. per Kg., respectively.

Analysis [1]

The observations, with the exception of those made under cortisone therapy, were subjected to a covariance analysis. The regression of urine flow on solute excretion did not change significantly between patients, or with thyroid therapy ($p > 0.20$). For all the values, the regression line, shown in Figure 6, was highly significant ($p < 0.002$). The urine flow values observed under different conditions, when adjusted for differences in solute excretion, were not significantly different from each other ($p > 0.10$).

The regression equation is:

$$Y = 0.148 + 1.484\,X$$

where Y = urine flow in ml. per minute,
X = solute excretion in mOsm. per minute.

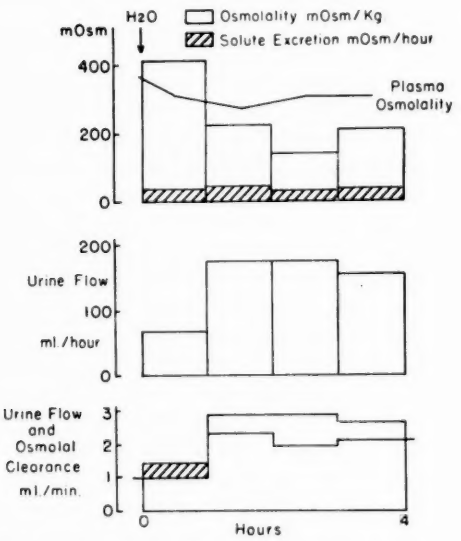

FIG. 5. CASE 2: THE RESPONSE TO WATER LOADING ON THYROID AND INTRAMUSCULAR CORTISONE, 5 MG. TWICE DAILY

The mode of presentation is as in Figure 4.

[1] The statistical analysis was made by Dr. Mindel C. Sheps, Department of Preventive Medicine, Harvard Medical School.

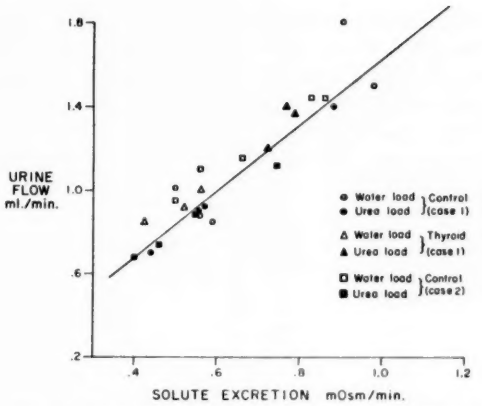

FIG. 6. THE RELATIONSHIP BETWEEN SOLUTE EXCRETION AND URINE FLOW IN THE ABSENCE OF CORTISONE ADMINISTRATION IN THE TWO PATIENTS WITH COMBINED ANTERIOR PITUITARY AND NEUROHYPOPHYSEAL FAILURE

The correlation is highly significant ($p < 0.002$).

Controls

The responses to water and urea loading are summarized in Figures 7 and 8. In anterior hypopituitarism the same failure of prompt water diuresis was noted as was present in the patients with combined anterior pituitary and neurohypophyseal deficiency. Urine flow never exceeded 1.33 ml. per minute and urine osmolalities remained approximately 600 mOsm. per Kg. Urine flow was a function of solute excretion. Only 22 per cent of the water ingested was excreted in four hours, confirming the findings of Burston and Garrod (17). Urea caused maximal concentration of the urine with osmolalities approaching the normal upper limit of 1,400 mOsm. per Kg.

The patient with uncomplicated diabetes insipidus excreted a water load promptly. Urine flow was high and urine osmolalities were about 100 mOsm. per Kg. When given urea after 14 hours dehydration, urine osmolalities rose to 420 mOsm. per Kg., indicating that a hypertonic urine can be elaborated in the absence of ADH.

The normal subject showed a wide range of renal concentrating and diluting capacity. Following water ingestion, maximal urine flow of

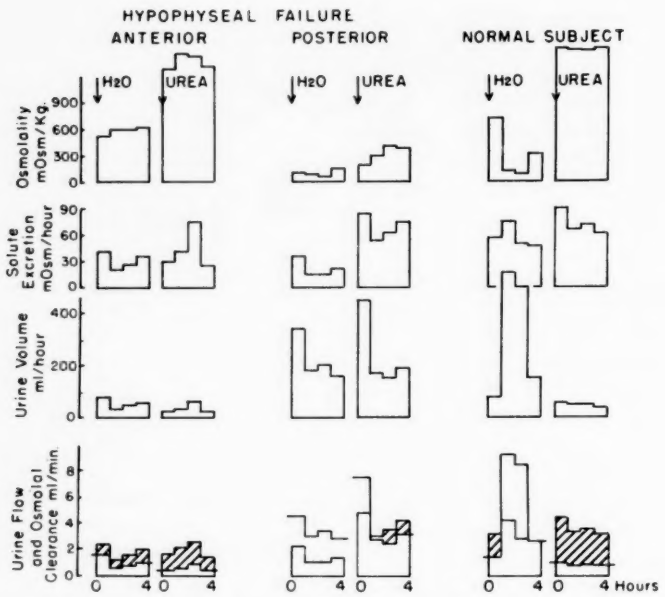

FIG. 7. THE RESPONSE TO ORAL WATER AND UREA LOADING OF THE CONTROL SUBJECTS–THE PATIENT WITH ANTERIOR HYPOPITUITARISM, THE PATIENT WITH POSTERIOR HYPOPITUITARISM AND THE NORMAL MAN

The mode of presentation is as in Figure 4.

9.3 ml. per minute was reached during the second hour. As urine flow increased, urine osmolality fell despite a rise in solute excretion. Following urea there was little change in urine flow and urine osmolality remained at the normal upper limit of 1,400 mOsm. per Kg.

DISCUSSION

A few patients with anterior pituitary and neurohypophyseal insufficiency have been reported in the literature (4–7), but only one case has been studied in detail. This patient, described by Leaf, Mamby, Rasmussen and Marasco (5), usually excreted a hypotonic urine even after prolonged fluid deprivation. She was capable of prompt water diuresis and showed no response to cortisone or ACTH. The authors concluded that the subject probably had enough anterior pituitary and adrenocortical function left to maintain the renal concentrating defect of neurohypophyseal failure, which was further complicated by a lesion of the thirst center.

In the two subjects of the present study the diagnosis of combined anterior pituitary and neurohypophyseal failure was based of necessity upon clinical evaluation. The most striking finding was the excretion of a hypertonic urine of relatively fixed osmolality and inability to excrete a water load promptly despite marked changes in plasma osmolality. That the high urine concentration was caused by minimal secretion of ADH seems unlikely in view of the absence of any appreciable change following water as well as urea loading. Furthermore, the close correlation between urine flow and solute output would not have been present if the proportion of water reabsorbed from the glomerular filtrate had varied more than slightly. The increased urine volumes in Case 2 may suggest residual anterior pituitary function. Although this cannot be excluded, it was obviously inadequate to maintain the concentrating defect characteristic of uncomplicated neurohypophyseal insufficiency. Cortisone both corrected the incapacity for prompt water diuresis and "unmasked" the latent diabetes insipidus.

Changes in the glomerular filtration rate were

reflected in the urine concentration. In the absence of hormone administration, urine osmolality was high and glomerular filtration low, in accordance with other published data (4, 7, 17). Thyroid raised the glomerular filtration rate with a concomitant fall in urine concentration. When cortisone was added to the treatment in Case 1, renal plasma flow and glomerular filtration rate approached normal levels and urine isosmolal concentrations (Table II). It seems reasonable, therefore, to attribute the hypertonicity of the urine, at least in part, to the reduction in glomerular filtration rate with a diminution in the volume filtered in all nephrons rather than to a selective cessation of filtration in a group of nephrons. This interpretation is in accord with recent experimental evidence on the production of hypertonic urine by lowering the glomerular filtration rate in the absence of ADH (18, 19).

Prompt water diuresis with the establishment of free water clearance was still impaired in Case 1 (Figure 4, Experiment 5), despite a normal renal plasma flow of 527 ml. per minute and a glomerular filtration rate of 116 ml. per minute when the patient was on triiodothyronine, 20 μg. three times a day, and cortisone, 37.5 mg. daily (Table I). Only after 100 mg. cortisone was given preceding the test was free water clearance present, presumably because the smaller dose of cortisone had lost its effectiveness by the end of four hours when the water was ingested (20).

In Case 2, free water clearance was present on thyroid and cortisone therapy at both dose levels. The excretion of only 50 per cent of the water load is believed to have been due to dehydration, as the experiments were carried out during a heat wave. With adequate water ingestion, this patient achieved a urine flow in excess of 10 ml. per minute, preceding the saline test.

The effect of cortisone in restoring the capacity for free water clearance cannot be ascribed to increased breakdown of protein and provision of urinary solute as urea, since urea administration had no such effect. It seems well documented (18, 19) that a lowered glomerular filtration rate is associated with impaired water diuresis, but restoration of the glomerular filtration rate in Case 1 to normal did not appear to correct the defect in accord with other data (21, 22). It is suggested, therefore, that cortisone, in addition to restoring the glomerular filtration rate toward normal levels, may bring about an intrinsic change in the renal tubular handling of water, possibly as a reflection of a generalized cellular effect. That steroids can affect the renal tubular reabsorption of water has been shown (23). The hypothesis is advanced that the presence of adrenocortical hormone is required in addition to a normal glomerular filtration rate for the production of free water clearance. Whether steroids effect a re-

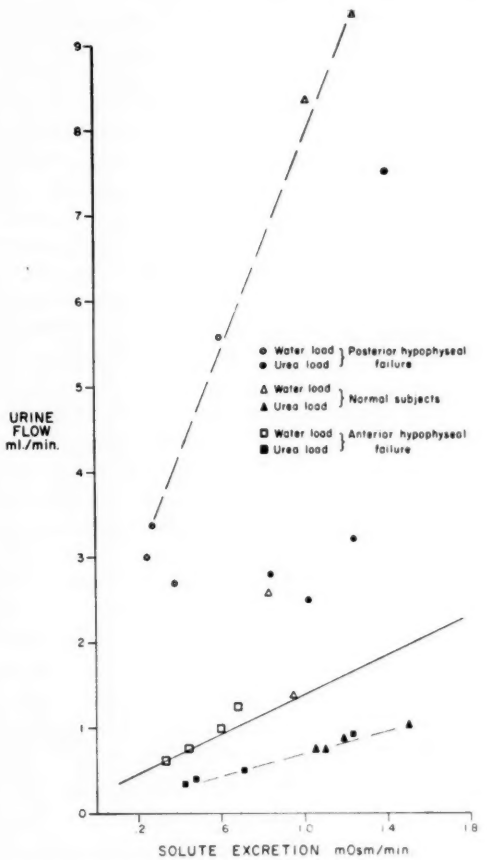

FIG. 8. THE RELATIONSHIP OF URINE FLOW TO SOLUTE EXCRETION IN THE CONTROL SUBJECTS—THE PATIENT WITH DIABETES INSIPIDUS, THE PATIENT WITH ANTERIOR PITUITARY INSUFFICIENCY AND THE NORMAL MAN

The continuous line corresponds to the regression line in Figure 6. The broken lines indicate the extreme ranges of concentration and dilution met within the present investigation.

distribution of solute absorption between the proximal and distal tubule or an alteration in the permeability of the renal tubular epithelium to water cannot be deduced from the present findings and further studies in pituitary and adrenocortical insufficiency are needed.

The normal subject illustrated the full range of physiological adaptation to water and solute loading.[2] Only one extreme of this range was attained by the patient with anterior hypopituitarism, who was able to elaborate a highly concentrated urine, but not a dilute urine. Only the other extreme was reached by the patient with diabetes insipidus who alternately was able to elaborate a hypotonic urine but had lost normal concentrating capacity. The production of a hypertonic urine in this patient in response to dehydration and urea loading was most likely the result of a reduction in the glomerular filtration rate. The patients with both anterior pituitary and neurohypophyseal insufficiency had lost the capacity for maximal concentration and dilution and were able to alter renal excretory function only within the narrow range imposed by the limited variability of the glomerular filtration rate. That the lack of prompt water diuresis in anterior hypopituitarism is the result of a relative or absolute ADH excess (24, 25) seems most unlikely in view of the persistence of the defect in the present cases with absent neurohypophyseal function and similar cases reported by others (4, 6, 7). A comparison of the findings in the various cases clearly indicates the range of activity and the absence of any interrelationship of ADH and adrenocortical hormones. Adrenocortical steroids restored the ability for free water clearance independent of the functional integrity of the neurohypophyseal antidiuretic mechanism. That the normal action and release of ADH is not interfered with by adrenocortical steroids has previously been demonstrated (26).

SUMMARY AND CONCLUSIONS

Studies are reported in two patients with space occupying lesions in the region of the sella turcica in whom a combined deficiency of anterior pituitary and neurohypophyseal function seems reasonably certain. Renal plasma flow, glomerular filtration rate and the responses to water and urea loading have been examined before and after hormone administration. In one patient total body water and extracellular fluid volume were also measured.

In the absence of cortisone the urine was hypertonic at all times and urine flow was a function of solute excretion. Cortisone raised the glomerular filtration rate to normal and restored the capacity for free water clearance. It is suggested that cortisone, in addition to raising the glomerular filtration rate, may also affect renal tubular water transport because prompt water diuresis was still impaired in one case on inadequate cortisone replacement despite a normal renal plasma flow and glomerular filtration rate.

A comparison of the data obtained in patients with combined defects and those in patients with diabetes insipidus and anterior hypopituitarism suggests that antidiuretic hormone (ADH) and cortisone exert their effects independently.

[2] The high urine osmolality obtained with urea was due to the absence of osmotic diuresis. Urea is a poor diuretic solute because of its large volume of distribution and moderate tubular reabsorption.

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STUDIES OF THE EFFECT OF DIETARY PROTEIN AND CALORIC LEVELS ON THE KINETICS OF NITROGEN METABOLISM USING N^{15} L-ASPARTIC ACID

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There have been relatively few attempts to study the dynamic aspects of nitrogen metabolism from the standpoint of the intact overall organism. The complexity of the problem precludes an exact mathematical description at present, but models which purport to enable one to compute values for nitrogeneous metabolic pool size and rate of incorporation of nitrogen into the human have been devised by San Pietro and Rittenberg (1) and Olesen, Heilskov and Schoenheyder (2). The approach of San Pietro and Rittenberg was to devise a model on biologic grounds and describe the model by equations, whereas that of Olesen and co-workers was to derive a model from experimental data which could be described by certain equations. The latter, by extending the length of time of the experiment, changed the nature of some of the necessary assumptions of the San Pietro-Rittenberg type model. While there are certain advantages and disadvantages inherent in both types of approach, it would appear that the San Pietro-Rittenberg approach is as satisfactory as any yet available.

The purpose of this study of the San Pietro-Rittenberg model was to determine: *1*) the reproducibility of calculated values for nitrogenous metabolic pool size (P), rate of nitrogen incorporation into the protein of the organism (S), and rate constant for metabolic pool turnover (B) in one individual under the same experimental conditions, and *2*) the effects of variation of caloric and protein content of the diet on the calculated values. Further mathematical manipulations of the original equations have led to an expression which allows the computation of B at any time, thus providing further insight into the model.

METHODS AND MATERIALS

The patient was a 53 year old white female with histologically proven lymphosarcoma of three and one-half years duration. She had been treated with radiotherapy on a number of occasions, the last course of treatment having been completed six weeks before admission. At the time of admission her spleen was palpable one finger-breadth below the costal margin, but there was no significant adenopathy or other evidence of lymphosarcoma. During the 84 days of the study there was no evidence of tumor growth and only a slight weight change. She was afebrile during all eight studies. Radioactive iodine uptake and protein bound iodine determinations were in the lower range of normal.

Metabolic balance studies were performed during her entire hospitalization (see Figure 1) by methods previously described (3). Caloric expenditure determinations were performed by the technique of Johnson and Newburgh (4). Four pairs of duplicate studies of the model were performed on four different diets. Diet I consisted of 1,800 calories with 40 Gm. of protein; Diet II contained 1,200 calories and 40 Gm. of protein; Diet III contained 1,200 calories and 80 Gm. of protein; and Diet IV, 1,800 calories and 80 Gm. of protein. Two studies were performed on each diet, the first after eight days of equilibration with the diet and the repeat study eight days later. The first study on Diet I was performed after 12 days of equilibration. Each study was performed after the oral administration with breakfast of 100 mg. of excess N^{15} as L-aspartic acid (1.508 Gm. of 63.0 atom per cent excess N^{15} L-aspartic acid for Studies 1 through 6, and 0.995 Gm. 95.5 atom per cent excess N^{15} L-aspartic acid for Studies 7 and 8). L-Aspartic acid was prepared by the method of Wu and Rittenberg (5). Urine was collected at 20 minute intervals for the first four hours, at 30 minute intervals for about the next three hours, and at wider intervals thereafter (Table II). The specific activity curve of urinary urea was determined on urea isolated by xanthydrol precipitation (6). Urea excretion was determined by the urease method followed by Nesslerization (7). The "urea pool" was measured on two occasions (Studies 1 and 4) by body water determination using tritium oxide[1] (8). Total nitrogen excretion was determined by the Kjeldahl method (9).

[1] The authors wish to thank Drs. N. Berlin and D. Nathan for the body water determinations, and Mr. A. McNish for mass spectrometer determinations.

RESULTS AND DISCUSSION

The problem of understanding the model and its limitations is that of relating the mathematics to biological meaning. Because of the limitations inherent in the assumptions on which the mathematics is based, the values of B (rate constant for metabolic pool turnover), P (metabolic pool size as grams of N), and S (the grams of N incorporated into the protein of the organism per day) undoubtedly do not represent the true values of these parameters of nitrogen metabolism as originally defined. The exact meaning of the computed values cannot be defined, but it seems probable that the values for P and S do not represent the entire body, but only a portion, *i.e.*, its more dynamically active constituents. Information pertaining to these constituents, however, may be of equal or perhaps greater importance than that pertaining to the entire body. While the calculated value for P is quite similar to the product of extracellular free amino acid nitrogen and body water, a more correct value of P is probably greater than this because of the significantly higher intracellular concentration of amino acids.

Stated in biological terms, there are six major assumptions upon which the equations of the San Pietro-Rittenberg model are based. The assumptions inherent in the model are:

1) There is one nitrogenous metabolic (P) and one urea (U) pool in the body, or there are X pools in sufficiently rapid equilibrium to be considered as single pools.

2) The size of the nitrogenous metabolic pool (P) and the urea pool (U) are constant during the course of the experiment and urea and total nitrogen are excreted at a uniform rate during the day. It is important to realize that the assumption of P and U being constant is only necessary "during the course of the experiment." The measurements involving isotopes which are used for the solution of Equation 16 (1) for B and then the solutions of P and S are completed in about five to six hours, although the non-isotopic measurements (E_u and E_t)[2] are over a 24 hour period. It is probably safe to assume U to be a constant. Whether there is significant variation in P during the experiment is unknown, but it seems likely that there is some temporary increase in P following the ingestion of a meal. In previous studies (1) the dose of nitrogen given as isotopic amino acid was significant relative to the computed size of the metabolic pool, whereas in the present studies it varied between 4.0 and 9.7 per cent of the computed size of the metabolic pool. The question arises as to whether nitrogen equilibrium is necessary for the model to be valid. It should be noted that D (grams of dietary nitrogen per day) appears nowhere in the equations used to calculate B, P and S. If the basic assumption is made that P is a constant during the course of the experiment, then the model would theoretically be valid for positive and negative nitrogen balance for the following reasons: Stating that P is a constant is equivalent to stating that $D + R = S + E_t$.[3] The assumption of P being a constant eliminates any use of the diet as an individual variable, since it is only important for $D + R = S + E_t$, which in turn must be constant during the experiment. The case of nitrogen equilibrium is merely where $D = E_t$ and $S = R$. In positive nitrogen balance $D > E_t$ and $S > R$, but if P is constant, then $D + R$ still $= S + E_t$. F[4] would presumably be increasing by a value of $D - E_t$ or $S - R$. If one assumes that P is only constant during nitrogen equilibrium, then the model is theoretically valid only for nitrogen equilibrium. The patient reported in the present study was very close to nitrogen equilibrium in the last four studies (within 0.25 Gm. N per day on Studies 5, 6 and 7, and 0.5 Gm. N per day on Study 8) (Figure 1) and in slightly negative nitrogen balance in the first four (1 Gm. N per day).

3) N^{15} after administration is instantaneously distributed throughout the metabolic pool in the same distribution pattern as unlabeled nitrogen. This is intimately related to Assumption 1. When a single labeled amino acid is used (as was done here), this distribution of isotope throughout the metabolic pool involves three separate processes: *a*) the distribution of N^{15} among the other amino acids of the metabolic pool by trans-

[2] E_u is the number of grams of urea nitrogen excreted per day; E_t is the number of grams of total nitrogen excreted per day.

[3] D is defined as the grams of dietary nitrogen per day; R is the number of grams of nitrogen returned to the metabolic pool per day through breakdown of protein.

[4] F is the number of grams of protein nitrogen in the body.

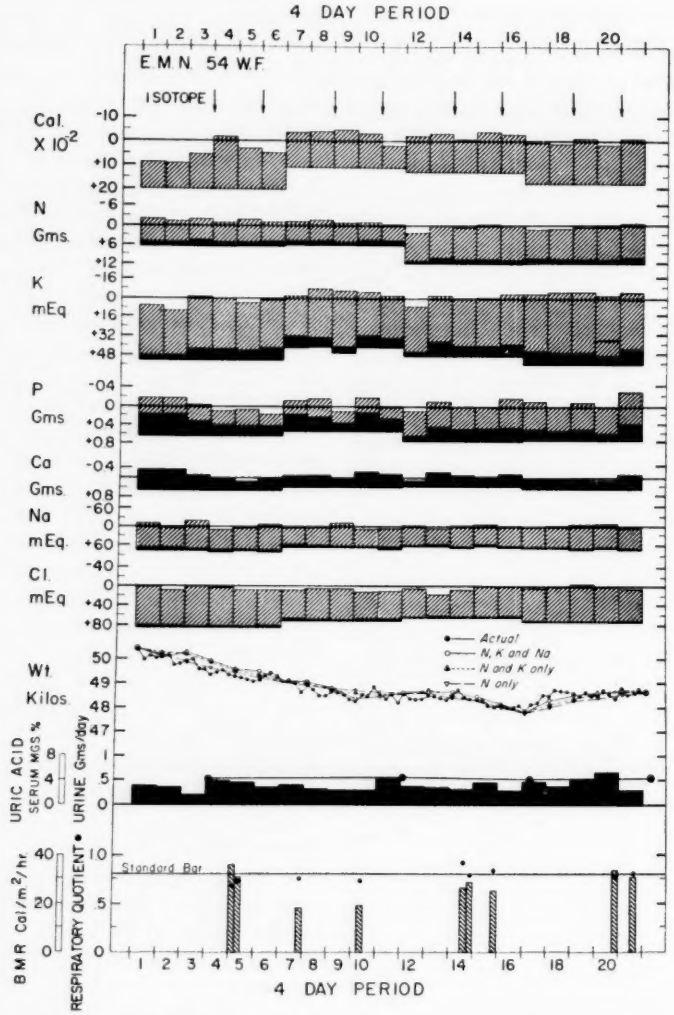

FIG. 1. METABOLIC BALANCES IN A 54 YEAR OLD WOMAN WITH CLINICALLY INACTIVE LYMPHOSARCOMA ON VARIOUS DIETS

The first seven bar graphs in the chart present balance data according to the general scheme proposed by Reifenstein, Albright and Wells (14). Intake is plotted downward from the zero line; output in terms of feces and urine is then plotted upward from the point representing intake. When total output touches the zero line, equilibrium or balance is represented; when output fails to reach the zero line, positive imbalance is represented; and when output extends beyond the zero line, negative imbalance is represented. The ordinates of the nitrogen, potassium, phosphorus and calcium graphs are so selected that equal heights represent the approximate ratios at which these elements exist in normal protoplasm and bone, *i.e.*, 3 Gm. of nitrogen to 8 mEq. of potassium to 200 mg. of phosphorus (protoplasm) and 400 mg. of calcium to 200 mg. of phosphorus (bone). The ordinates of the sodium and chloride graphs are so selected that equal heights represent the approximate ratios at which these elements exist in normal extracellular fluid, *i.e.*, 150 mEq. of sodium to 100 mEq. chloride.

Below the bar graphs, line graphs record the patient's weight. The solid line connecting the closed circles records the actual weight measured daily. The solid line connecting the open circles depicts the theoretical weight based on considerations of the gain or loss of intracellular and extracellular fluid quantified by balances of nitrogen, potassium and sodium. The broken line joining the closed triangles depicts the theoretical weight based on considerations of gain

amination, *b*) mechanical mixing of these labeled amino acids from one organ to another, and *c*) entrance into cells. This assumption of instantaneous distribution of N^{15} throughout the metabolic pool is incorrect because of these considerations. The first objection (the finite time involved in transamination) could be partially overcome by using a mixture of labeled amino acids such as that obtained from hydrolysate of labeled protein. Thus, transamination of the isotope would have occurred prior to its administration to the patient. Intravenous administration of the isotope would tend to decrease the error of mechanical mixing. Since the liver removes a large fraction of the administered dose of labeled amino acid, it is probable that the values computed from the model are to a large extent a reflection of liver activity.

4) Nonurea nitrogen which is excreted (denoted by E_x) comes directly from the metabolic pool. This is true with several exceptions such as uric acid and creatinine, which are not really components of the metabolic pool as defined here. Examination of the equations reveals that the compartment of origin of E_x does not effect the equations or the computed values, except for the computation of S, where E_t is subtracted from BP. The calculated value of S is the total amount of nitrogen leaving the metabolic pool (BP), minus that excreted from the metabolic pool into the urine (assumed in the model to equal E_t, but actually somewhat less than E_t because certain components of E_t such as uric acid and so forth are not excreted from the metabolic pool as defined here). This error tends to decrease the value of S somewhat below the true value. The above discussion pertains when S is defined as the total amount of nitrogen incorporated into the organism per day. This would include nucleic acids and other nonprotein materials. If S is defined as the grams of nitrogen incorporated into protein only, then this assumption introduces much less error, because the computed value of S would not include these nonprotein materials. The subtraction of the nonmetabolic pool component of E_x from BP would decrease S by a value equal to the nonprotein nitrogen incorporated in the steady state.

5) The amount of isotope returned to the metabolic pool from protein, which during the course of the experiment had incorporated N^{15}, is negligible. This assumption corresponds to neglecting the term $Q\,dt$ in Equation 1 (1). Since the crucial isotope measurements are made in about six hours, it is not difficult to accept this assumption on the basis of present day knowledge. From the data of San Pietro and Rittenberg (1) in Experiment DR-1, the value of the integral of $Q\,dt$ over a six hour period is roughly estimated as about 5 per cent of the administered dose of isotope $\left(\frac{S + E_u}{E_u} \times \frac{3.7}{4}\right)$. This is based on the assumption that the integral of $Q\,dt$ is a linear function of time, which is

or loss of intracellular fluid quantified by balances of nitrogen and potassium. The broken line joining the open triangles depicts the theoretical weight based on considerations of gain or loss of intracellular fluid based on nitrogen balance alone.

The line graph below the weight curves represents the serum uric acid level and the bar graph, the daily excretion of uric acid.

The bar graphs at the bottom of the chart represent the patient's basal metabolic rate and the closed circles the respiratory quotient on selected days during the study (15). The line labeled "standard bar" represents the Aub-Dubois standard basal metabolic rate for a woman of the age and with the surface area of this patient and also the respiratory quotient of a patient consuming an average mixed diet.

The Arabic numerals along the top and bottom of the chart refer to the four day metabolic balance periods of the study and may be identified with the isotope studies and diets by reference to the arrows at the top of the chart and to Tables I through V.

Note that low protein intake at both calorie levels was associated with weight loss and negative nitrogen imbalance. High protein intake was associated with nitrogen equilibrium at both levels of caloric intake, but was accompanied by caloric equilibrium and weight gain only when the caloric intake was high. The calcium balances suggest more retention of calcium on the high calorie, high protein, than on other regimens.

The actual and theoretical weight curves are practicably superimposable on one another. Uric acid excretion rose when calorie intake was increased while the patient ingested a high protein intake.

The basal metabolic rate declined dramatically on the low calorie, low protein regimen, rose on the high calorie, low protein, and returned to standard when the calorie and protein intake were both high. The respiratory quotient was lower on low than on high protein regimens.

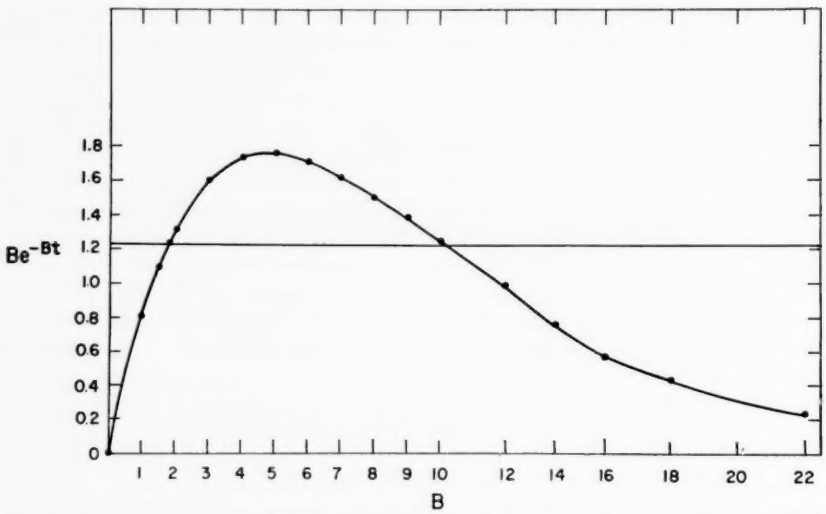

FIG. 2. THE CURVE OF THE EXPRESSION Be^{-Bt} AS A FUNCTION OF B WHERE t EQUALS 0.208

only an approximation. While this is not completely negligible, it introduces no great error over this period of time.

6) The overall processes involved in the model can be described by first order kinetics. This problem has been discussed by Reiner (10, 11).

The rate constant for metabolic pool turnover (B) can be computed in several ways. Before discussing these methods it should be pointed out that the model assumes B to be a constant since $B = \frac{E_t + S}{P}$ where E_t, S and P are assumed to be constants. San Pietro and Rittenberg solve for B by equating the derivative of the expression for isotope concentration in the urea pool to zero at the time of maximum isotope concentration. This leads to the expression $\frac{E_u}{U} e^{-(E_u t\ max/U)} = B\, e^{-Bt\ max}$. Casual examination would suggest that this is an identity in which $B = \frac{E_u}{U}$, which would appear to be a contradiction of the original definition of B. It is readily seen however, as shown in Figure 2, that for a given value of t max (0.208 was used), when $\frac{E_u}{U} e^{-(E_u t\ max/U)}$ is plotted as a function of B, there are two values of B which satisfy a given value (1.23 for Study 2) of $\frac{E_u}{U} e^{-(E_u t\ max/U)}$ below the maximum point of the curve. One of these is $\frac{E_u}{U}$, the other being the value sought here. Figure 2 demonstrates the values of B which satisfy the conditions in Study 2 where t max = 0.208 days and $\frac{E_u}{U} = 1.805$.

Thus, the San Pietro-Rittenberg method allows the computation of B at only one time point during the experiment since the equation used for this computation is true only at the time of maximum specific activity of urea. An equation which enables one to compute B at any time (provided data for twice that length of time are available) can be derived from the equation for the concentration of isotope in the urea pool as follows:

C_u (as atom per cent excess, where λo = grams of N^{15} excess given at t = 0) =

$$\frac{100\,\lambda o\, E_u}{P\,(E_u - BU)} (e^{-Bt} - e^{-(E_u t/U)}).$$

Let

$$\frac{100\,\lambda o\, E_u}{P\,(E_u - BU)} = x, \quad e^{-B} = y \quad \text{and} \quad e^{-E_u/U} = D.$$

Also let

$$t_2 = 2\,t_1 \quad \text{and} \quad \begin{matrix} C_u\,(t_1) = C_{u1} \\ C_u\,(t_2) = C_{u2}. \end{matrix}$$

Then

$$C_{u1} = x\,(y^{t_1} - D^{t_1})$$

$$C_{u2} = x\,(y^{t_2} - D^{t_2}) = x\,(y^{2t_1} - D^{2t_1})$$

$$\frac{C_{u2} + x\,D^{2t_1}}{(C_{u1} + x\,D^{t_1})^2} = \frac{x\,y^{2t_1}}{x^2\,y^{2t_1}} = \frac{1}{x}$$

$$C_{u2}\,x + x^2\,D^{2t_1} = C_{u1}^2 + 2\,x\,D^{t_1}\,C_{u1} + x^2\,D^{2t_1}$$

$$x = \frac{C_{u1}^2}{C_{u2} - 2\,C_{u1}\,D^{t_1}}.$$

Now

$$y^t = \frac{C_u}{x} + D^t$$

$$y^{t_1} = \frac{C_{u1}}{x} + D^{t_1} + \frac{C_{u2}}{C_{u1}} - D^{t_1}.$$

But $B = \dfrac{-\ln y^t}{t}$, thus at t_1

$$B = \frac{-\ln\left(\dfrac{C_{u2}}{C_{u1}} - D^{t_1}\right)}{t_1}$$

$$= \frac{-\ln\left(\dfrac{C_{u2}}{C_{u1}} - e^{(-E_u t_1/U)}\right)}{t_1}$$

where $\dfrac{C_{u2}}{C_{u1}}$ represents the ratio of isotope concentrations in the urea pool at any two times which are in a ratio of 2 to 1. All the assumptions of the model (that B is a constant and so on) are inherent in the above equation for B at any time t_1, since this equation is derived entirely from the equation for C_u from the model. If the assumptions on which the model is based were absolutely correct, the computed values of B plotted at different times would produce a straight line of zero slope, *i.e.*, a constant. B was computed at various time intervals in all eight studies.

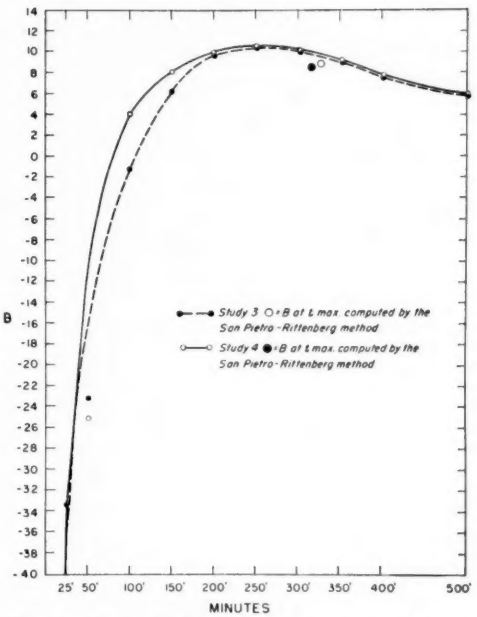
B
MINUTES
25' 50' 100' 150' 200' 250' 300' 350' 400' 500'
Study 3 ○+B at t max. computed by the San Pietro-Rittenberg method
Study 4 ●+B at t max. computed by the San Pietro-Rittenberg method

FIG. 3. COMPUTED VALUES OF B AT VARIOUS TIMES DURING STUDIES 3 AND 4

The general shape of the curve from the duplicate Studies 3 and 4 is demonstrated in Figure 3. The values for the other studies, where the agreement of duplicate curves was not quite so remarkable, is shown in Table I. The computed value of B starts very early as a large negative value, increases rapidly to a positive value, reaches a maximum and gradually declines. Computation of B at various times from the

TABLE I

Computed values of B at different times

Study		1	2	3	4	5	6	7	8
Time after administration of isotope									
min.	*days*								
25	0.0174	−80.2	−96.2	−33.3	−61.1	−24.72	−26.73	−18.6	−30.53
50	0.0348	−18.6	−31.7	−23.3	−25.1	−35.2	−19.74	− 3.83	−32.7
100	0.0696	− 1.42	−25.9	− 1.19	4.11	0.273	1.98	− 2.57	− 0.14
150	0.1041	6.03	6.32	6.17	8.05	5.41	7.53	9.58	15.33
200	0.1394	10.86	8.54	9.58	9.88	8.96	11.16	13.27	17.26
250	0.1736	11.76	9.54	10.31	10.47	13.1	12.16	13.34	21.03
300	0.2082	9.92	9.78	10.00	10.18	13.6	9.93	10.02	22.64
350	0.2431	8.0	9.04	9.04	9.12	10.18	7.78	7.75	16.53
400	0.278	6.52	8.44	7.56	7.75	7.00	6.42	6.42	9.88
500	0.347	5.18	7.39	5.99	5.90	4.08	5.04	4.69	5.18

TABLE II

Specific activity of urinary urea *

Study 1		Study 2		Study 3		Study 4		Study 5		Study 6		Study 7		Study 8	
Time	Atom % excess	Time	Atom % excess	Time	Atom % excess	Time	Atom % excess	Time	Atom % excess	Time	Atom % excess	Time	Atom % excess	Time	Atom % excess
min.		*min.*		*min.*		*min.*		*min.*		*min.*		*min.*		*min.*	
27	.0262	10	.0375	10	.0240	10	.0387	10	.0214	10	.0347	10	.0294	10	.0365
65	.1911	34	.0261	30	.0511	30	.0501	30	.0424	30	.0606	30	.0522	30	.0365
83	.2639	59	.1048	50	.1050	50	.1345	50	.0716	50	.1406	50	.0561	50	.0922
102	.3620	80	.1970	70	.2206	70	.2603	70	.1595	70	.2492	70	.1272	70	.1714
125	.4459	100	.3046	90	.3132	90	.3760	90	.2560	90	.3218	90	.2396	90	.2967
146	.5425	120	.3969	110	.4173	110	.5022	110	.4038	110	.4597	110	.3924	110	.4287
169	.6160	140	.4774	130	.4821	130	.5599	130	.4824	130	.5155	130	.4888	130	.5302
192	.6882	160	.5405	150	.5480	150	.6098	150	.5164	150	.5921	150	.5355	150	.6608
211	.7186	180	.6009	170	.6248	170	.6615	170	.5335	170	.6376	170	.6481		
232	.7475	200	.6255	190	.6658	190	.6988	190	.5839	190	.6683	190	.6591	190	.6813
252	.7512	225	.6356	210	.7003	210	.7476	210	.6139	210	.7013	210	.6598	210	.6934
275	.7568	255	.6800	230	.7068	230	.7699	230	.6307	230	.7141	230	.6710	230	.6861
305	.7455	285	.6930	255	.7114	250	.7416	250	.6804	250	.7191	250	.6814	250	.6449
340	.7124	315	.6929	285	.7300	270	.7824	270	.7024	270	.7277	275	.6532	275	.6580
396	.6699	345	.6982	315	.7473	290	.7806	290	.7041	295	.7195	305	.6277	305	.6337
466	.6437	375	.6955	345	.7354	315	.7995	310	.6854	325	.6854	335	.6117	335	.6094
590	.6093	550	.6101	375	.6954	345	.7964	330	.7043	355	.6539	365	.5721	365	.5903
720	.3726	1,070	.3628	545	.6158	375	.7785	350	.7006	385	.6037	395	.5739	395	.5520
				738	.5047	405	.7488	370	.6503	415	.5892	425	.5044	425	.5218
				1,097	.3494	567	.6633	395	.6141	445	.5747	455	.4955	455	.4836
						1,075	.4191	425	.5931	475	.5490			595	.4094
								455	.5355	602	.4829	1,080	.2395	1,080	.2433
								485	.5424	1,075	.2685				
								605	.4928						
								1,842	.2950						

* The specific activities are given at the mid-points of the urinary collection periods.

published data of San Pietro and Rittenberg (1) and Crispell (12) produces similar curves.

The fact that the computed values of B vary during the course of the experiment, as they do when the experimental data are used to compute B at different times, means that the experimental data do not fit the equations of the model early in the experiment. This is a reflection of the fact that some of the assumptions on which the equations of the model are based are somewhat incorrect. The t max (time of maximum urea specific activity) occurs at a time when there is not great deviation of B from linearity with zero slope, thus demonstrating that the use of t max for computation of B is justified.

The erroneous assumption which explains the early negative value of B is that of instantaneous mixing of N^{15} throughout the metabolic pool. If this assumption were correct, λp would be expressed by a curve of the exponentially decreasing type with no ascending limb. The slope of this type of curve is negative throughout and equal to $-B\lambda p$. B for this type of assumed curve has a constant positive value. Experimentally, however, the curve of λp has an ascending limb and a descending limb, where the ascending limb corresponds to the period when isotope is entering P more rapidly than it is leaving. The slope prior to the maximum value of λp is positive and corresponds to the period when B is negative which gives a positive value to the expression $-B\lambda p$. The time at which $B = 0$ is the time of maximum value of λp. Thereafter the positive value of B corresponds to the negative slope of the curve of λp. The fact that B decreases gradually after reaching a maximum value is a reflection of the fact that the specific activity curve of λp decreases less rapidly after B max than the model would predict because of nonhomogeneity of the metabolic pool, in addition to the fact that isotope is entering the metabolic pool from various sources.

TABLE III

Measurements used in computing B, P and S *

Study	Diet	E_u	E_t	U
1	I	5.16	6.32	2.46
2	I	3.75	6.4	2.19
3	II	4.85	5.17	2.46
4	II	4.19	5.22	2.43
5	III	7.66	8.98	3.51
6	III	8.34	10.2	2.97
7	IV	8.73	8.98	2.97
8	IV	9.10	9.68	3.51

* E_u is the number of grams of urea nitrogen excreted per day; E_t is the total grams of nitrogen excreted per day; and U is the grams of urea nitrogen in the body.

TABLE IV

Values of B, P and S as computed from the time of maximum specific activity of urea *

Study	Diet	B	P	S	BP
1	I	10.5	1.77	12.3	18.6
2	I	10.36	1.65	10.7	17.1
3	II	8.85	1.94	12.0	17.2
4	II	8.95	1.63	9.40	14.6
5	III	9.00	2.18	10.6	19.6
6	III	9.05	2.52	12.6	22.8
7	IV	10.00	2.60	17.0	26.0
8	IV	14.24	1.80	15.9	25.6

* B is defined as the rate constant for metabolic pool turnover and is expressed in units of $days^{-1}$. P is the number of grams of nitrogen in the metabolic pool. S is the number of grams of nitrogen incorporated into protein per day. BP is the turnover rate of the metabolic pool expressed as grams of nitrogen leaving the metabolic pool per day.

The specific activities of urinary urea for all eight studies are presented in Table II and the data on urea excretion, total nitrogen excretion and urea pool size are presented in Table III. The values for B, P and S for all eight studies as computed by the San Pietro and Rittenberg method (1) are presented in Table IV. For Studies 1 through 6, the reproducibility of the values of B in duplicate studies is excellent. Reproducibility of P and S is generally within 20 per cent of the larger value. The accuracy of determination of the time of maximum isotope concentration in urea (t max) is important, since the computed values of B and P are relatively sensitive to small changes in t max as compared to E_u and U. Thus, in Study 8, a decrease of 10 minutes in t max (from 210 to 200 minutes) increases B from 14.24 to 15.3 and decreases P from 1.80 to 1.69. In the present studies, the frequency of determinations of urea specific activity (every 20 minutes) enabled us to determine accurately the t max.

It could be argued that since errors inherent in the assumptions tend to decrease the value of B, the most accurate value of B is the maximum one. Using the maximum value of B from the computed curve of B as a function of time and the time corresponding to this value of B, the

TABLE V

*Values of B, P and S from the maximum value of B as a function of time**

Study	Diet	B	P	S	BP
1	I	11.8	1.64	13.0	19.3
2	I	9.8	1.73	10.6	17.0
3	II	10.3	1.77	13.1	18.3
4	II	10.4	1.46	9.9	15.1
5	III	13.9	1.56	12.6	21.6
6	III	12.2	2.06	15.0	25.2
7	IV	14.0	2.18	21.6	30.6
8	IV	22.7	1.18	17.0	26.7

* B, P, S and BP are defined in Table IV.

values of P and S have also been computed as shown in Table V. Again with the exception of Study 8, the reproducibility of computed values is good.

Concerning the model in general, it is clear that the experimental data do not fit the equations of the model, particularly during the early part of the experiment (as shown by the variability of B computed at different times or by other methods) because of the errors inherent in the assumptions. The computed values, although erroneous in terms of their original definitions, are representative of these defined magnitudes. The relative constancy of the values in one patient under repeated study suggests that this method of approach measures the same thing in a particular individual from time to time. When the same individual is studied under different conditions, it seems probable that many of the errors in the model will "cancel each other out" allowing the effect of applied variables on the physiology of nitrogen to be detected readily. Previous studies using the model (12, 13) have produced data in accord with physiologic expectations.

Concerning dietary changes, the specific questions approached here were: *1*) What are the changes resulting from doubling the protein intake at two different levels of caloric intake? *2*) Are kinetic changes in the organism mediated through changes in rate constants, pool sizes or both? The present data, although of a preliminary nature, suggest that the rate constant (B) for metabolic pool turnover is relatively constant and unaffected by changes in both caloric and protein intake over the range of dietary variation and period of time studied (Table III). The metabolic pool size (P) and turnover rate (BP) appear to increase somewhat when the dietary protein is increased from 40 Gm. per day to 80 Gm. per day. The mean value of P in Studies 1 through 4 (Table IV) differed significantly from that of 5 through 8 at the 5 per cent level by the "t test." The rate of incorporation of nitrogen (S) (Table IV) appears to be unaffected by dietary changes until both nitrogen intake and caloric intake are raised (Studies 7 and 8). The mean value of S for Studies 7 and 8 differed significantly from that of Studies 1 through 6 by the "t test" at the 1 per cent level.

S did not change significantly when the per cent of protein in the diet was doubled with maintenance of constant caloric intake (Studies 5 and 6). If caloric expenditures were equal on Diets II and III [and measurements by the technique of Johnson and Newburgh (4) suggest that they were], then a greater fraction of the caloric demand was provided by protein on the higher protein diet (Diet III). This is to be expected and is in keeping with the increased urea and total nitrogen excretion observed on Diet III. The relative constancy of S on both diets also suggests that the additional protein was used to meet caloric needs and not for incorporation. It is well known that during starvation calories are furnished through breakdown of protein. Since synthetic processes require caloric expenditure, one would expect caloric needs to supersede to some extent synthetic processes. Here the individual maintained a steady state because the diet contained sufficient total calories including protein to meet caloric needs, some of which were derived from protein.

Since the total nitrogen excretion on Diets III and IV was the same, and the urea excretion was slightly greater on Diet IV (Table III), one might expect the same or slightly less nitrogen available for incorporation on Diet IV. The greater incorporation into protein (S) calculated therefore corresponds to more rapid turnover of the metabolic pool (BP) associated with the higher caloric intake.

Although S increased somewhat in Studies 7 and 8, the patient was in nitrogen equilibrium. This raises the question of whether high nitrogen and high caloric intake can increase protein turnover when there is no demonstrable increase in

the amount of protein in the body. It is of interest that this patient, after discharge from the hospital, gained 7 Kg. over the course of several months. The question also arises as to whether increased protein turnover is a prelude to positive nitrogen balance. Considerably more study will be necessary before these questions can be answered definitively.

SUMMARY

1. The mathematical model of nitrogen metabolism devised by San Pietro and Rittenberg (1) has been examined from the theoretical and experimental standpoint.

2. The reproducibility of calculated values for nitrogenous metabolic pool size (P), rate of incorporation of nitrogen into protein (S), and rate constant for nitrogenous metabolic pool turnover (B) in one individual under the same conditions is adequate enough to suggest that the effect of variables on the kinetics of nitrogen metabolism can be readily determined.

3. The effect of variation in both caloric and protein content of the diet was determined while the patient was on a metabolic balance study. The rate constant for nitrogenous metabolic pool turnover does not appear to vary significantly with the changes of diet studied here. Increased protein intake appears to increase the size of the nitrogenous metabolic pool (P) somewhat. The calculated rate of incorporation of nitrogen into protein (S) increased when both the caloric and protein intake were increased, even though the patient remained in nitrogen equilibrium. This raises the question as to whether increased caloric and protein intake can increase protein turnover prior to the onset of positive nitrogen balance.

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TOLBUTAMIDE HYPOGLYCEMIA IN ACUTELY DEPANCREATIZED DOGS * †

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The hypoglycemic activity of tolbutamide and related compounds is well established; however, their mode of action is still debated. Several theories have been postulated (1–5). One of the most widely held is that tolbutamide stimulates the beta cells of the pancreas and the resulting insulin secretion produces the fall in blood sugar (1). The reported ineffectiveness of tolbutamide in depancreatized animals (1) and humans (6) is taken as strong evidence for this theory. With few exceptions these studies were done sometime after recovery from the pancreatectomies. One of us (M.K.) in the past studied the possible pancreatropic effect of another substance, *i.e.*, anterior pituitary growth hormone (7). It was observed that growth hormone had a hypoglycemic effect in acutely depancreatized dogs but not in dogs several days after pancreatectomy. Accordingly, it was thought worthwhile to study the effect of tolbutamide in acutely depancreatized dogs.

METHODS

Adult male and female mongrel dogs were used. The animals were in the postabsorptive state. All the experiments were performed under sodium pentobarbital anesthesia. To assure complete removal of the pancreas the duodenum and pancreas were removed "en bloc." In the dog, the pancreas can be divided into three parts: a middle portion and two limbs. The middle portion is intimately adherent to that portion of the duodenum equivalent to the second, third and fourth parts of the human duodenum. The two "limbs," one to the right and the other to the left of the duodenum, are completely enveloped in omentum, well defined and easily mobilized. Since it is very difficult to remove completely and with certainty that part of the pancreas adherent to the duodenum and related structures, *i.e.*, bile duct, cystic artery, and so fourth, we resected the duodenum and attached pancreas as a whole. The bowel was cut at least one to two inches beyond the junction of duodenum and pancreas (through pyloric end of stomach and through jejunum). Since these were acute experiments no attempt was made to re-establish continuity of bowel. Once having mobilized and resected the duodenum and attached pancreas, the rest of the operation was relatively simple. Since both "limbs" of pancreatic tissue are free in omentum (except at one point, noted below) we could easily make our line of resection at least one-half inch away from, and parallel to the border of, the pancreas. The exception mentioned above is that the distal one-half to one inch of the left wing is closely applied to branches of the splenic artery and vein. At that point two or three tiny arteries and veins communicate between the vessels and pancreatic parenchyma. When these are ligated, the omentum containing the pancreas is easily stripped off the vessels. When the tip of the limb is mobilized the line of resection can be continued. In essence, then, the pancreas is removed intact and certainly completely. Early in the operative series, we did postmortem examinations. However, the difference between pancreas and omental fat is obvious enough at the time of operation to make postmortem examination unnecessary.

Blood samples were collected after administration of the anesthesia, immediately after removal of the pancreas and approximately every 30 minutes thereafter for four to seven hours. The blood glucose was determined by the Nelson-Somogyi method (8). Almost all of the blood specimens were taken from peripheral veins. On several occasions cardiac punctures were done, without deleterious effects. The sodium tolbutamide (2), 200 mg. per Kg., was dissolved in 15 to 20 ml. of normal saline and rapidly injected intravenously at the times noted.

RESULTS

Two unoperated dogs were given tolbutamide and the results paralleled those reported by others (9). In both cases the blood sugars fell approximately 60 per cent and the maximum effect was noted within two to four hours after injection.

Five dogs were subjected to pancreatectomy but did not receive tolbutamide (Table I and Figure 1). Within 90 minutes each dog's blood sugar reached hyperglycemic levels. The values then either stayed on a plateau for the next one to one and one-half hours or dipped to lower levels. Similar

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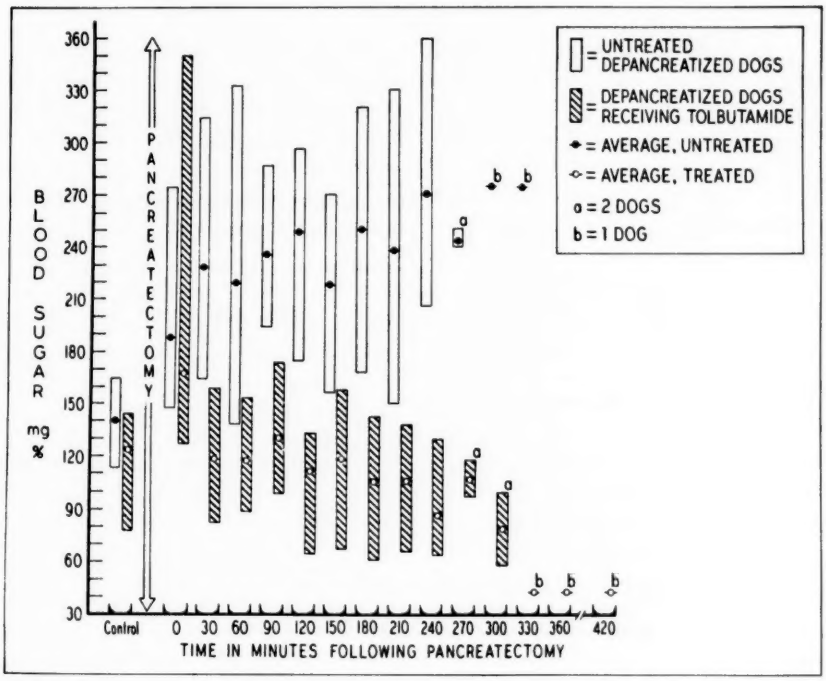

FIG. 1. COMPARISON OF THE BLOOD SUGARS OF UNTREATED DEPANCREATIZED DOGS WITH THOSE OF DEPANCREATIZED DOGS RECEIVING TOLBUTAMIDE

The tolbutamide was administered within 10 minutes of pancreatectomy. The top of each bar is equal to the greatest blood sugar value, at the time noted. The bottom of the bar is equal to the lowest blood sugar value.

TABLE I

The blood sugar curves of depancreatized dogs with and without tolbutamide

Dog	Blood sugar		Time in minutes												
	Control*	Zero time	30	60	90	120	150	180	210	240	270	300	330	360	420
			Blood sugar (*mg. %*)												
No.															
			Pancreatectomized dogs (no tolbutamide)												
1p	156	230	260	230	275	260	260	320	330	330					
2p	165	147	194	138	197	174	156	168	150	206					
3p	112	118	165	177	224	278	270	312	300	360					
4p	137	274	314	332	286	296	246	212	194	230	240	274	274		
5p	131	166	212	218	194	234	160	240	218	222	246				
			Pancreatectomized dogs (immediate tolbutamide)‡												
1p–T	78	95†	82	105	105	101	92	61	73	74					
2p–T	125	350†	134	110	173	127	158	142	129	96	118	100			
3p–T	132	147†	110	153	147	125	67	66		64					
4p–T	135	144†	159	121	150	124	150	118	100	130					
5p–T	131	127†	110	88	99	103	114	144	66	66					
6p–T	120	165†	85	125	133	133	133	133	128	72					
7p–T	144	142†	146		100	64	115		138	93	97	58	43	45	43

* Before pancreatectomy.
† After this specimen, tolbutamide given.
‡ Within one to 10 minutes after removal of the pancreas.

TABLE II

The effect of delayed tolbutamide administration on the blood sugar of depancreatized dogs

Dog	Blood sugar		Time in minutes											
	Control*	Zero time	30	60	90	120	150	180	210	240	270	300	330	360
			Blood sugar (*mg. %*)											
No.														
			Tolbutamide, 75 minutes after pancreatectomy											
8p-T	133	122		250†	182	131	128	175	186	161	163	150		
			Tolbutamide, 120 minutes after pancreatectomy											
9p-T	65	152		82		156*		209		222	152	176	225	287
10p-T	152	164		163		209*	222	180	221	209	213	193	209	197
11p-T	102	131		121		194*	173	135	137	96	59	94	80	80

* Before pancreatectomy.
† Time of administration of tolbutamide.

delayed declines of the blood sugar in depancreatized dogs have been noted previously (7). After approximately three hours, the blood sugar values started to rise again in those dogs in which a fall had occurred. At the end of the experiments, three and one-half to four hours after pancreatectomy, the blood sugars ranged from 206 to 360 mg. per cent.

The "control" blood sugars obtained in several dogs were higher than are usually found in fasting dogs. Increased adrenalin output in frightened, untrained animals is the most likely explanation. The marked hyperglycemia recorded within minutes of removal of the pancreas (zero time) in three dogs (1p, 4p and 2p-T) is also probably explained by excessive adrenalin secretion. It should be noted that the height of the blood sugar following pancreatectomy could not account for the difference in results between the untreated and treated dogs. Dog 2p-T had the highest zero time blood sugar, 350 mg. per cent, immediately after pancreatectomy and yet responded to the tolbutamide with a fall in blood sugar. Dog 11p-T which had a preinjection level of 194 mg. per cent also responded to the tolbutamide.

Seven dogs were given tolbutamide within two to 10 minutes after removal of the pancreas (Table I and Figure 1). Examination of the data reveals that during the first two hours the blood sugars did not rise as would normally be expected after pancreatectomy. Two to four hours after administration of the tolbutamide, the blood sugars started to fall. One dog (7p-T) was followed for seven hours and the hypoglycemic effect was found to persist throughout the period of observation. Figure 1 illustrates the difference between the treated and untreated depancreatized dogs. The blank bars represent the range of blood sugar values, at the times noted, for the untreated group. The cross-hatched bars depict similar values for the tolbutamide treated group. The average values for each set of readings are also noted. It is apparent that there is a striking difference in blood sugar values between the two groups. In every treated dog the last observed blood sugar, four to seven hours after pancreatectomy, was less than the preoperative (control) value. Final blood sugars ranged from 43 to 130 mg. per cent, a marked contrast to those seen in the untreated depancreatized dogs.

The administration of tolbutamide was delayed in four dogs. One, 8p-T, received the drug one hour after pancreatectomy and the others received it two hours after removal of the pancreas (Table II). Dog 8p-T showed a response in that five hours after pancreatectomy the blood sugar was only 17 mg. per cent higher than the preoperative value. The blood sugar curves of Dogs 9p-T and 10p-T do not seem to differ significantly from the depancreatized dogs not receiving tolbutamide. However, Dog 11p-T which, like Dogs 9p-T and 10p-T, received the drug two hours after pancreatectomy, showed a response similar to the dogs receiving the tolbutamide promptly after the pancreas was removed.

DISCUSSION

It is apparent that when tolbutamide is administered promptly after removal of the pancreas, a distinct hypoglycemic effect results. The blood sugars not only do not rise to the levels seen in untreated depancreatized dogs, but significant decreases in blood sugar occur. The decreases are comparable to those obtained in intact dogs receiving tolbutamide.

Fritz, Morton, Weinstein and Levine (10) have reported somewhat similar studies on three dogs and concluded that no effect occurred when the sulfonylureas (carbutamide) were administered to the acutely depancreatized dog. In our series of 11 dogs, those dogs exhibiting the most consistent effect were given the tolbutamide within 10 minutes of pancreatectomy. Fritz states that "carbutamide was administered 20 to 60 minutes after pancreatectomy." They state that no change was noted in the course of the blood sugar in the three hours that the dogs were followed. All of our dogs but one were followed for four hours or more. It is important to note that the effect became obvious in five of the seven dogs only at the fourth hour or later. One dog received tolbutamide 75 minutes after pancreatectomy and did not clearly show any effect at the end of three hours but did by the fifth hour.

Obviously, the hypoglycemic effect noted in our dogs was not mediated through the pancreas. However, these experiments do not clarify the relationship of insulin to tolbutamide activity. It is likely that the depancreatized dogs were not free of endogenous insulin and insulin may be a requirement for tolbutamide activity. The experiments in which the administration of tolbutamide was delayed were done to test this hypothesis. Turnover studies with labeled insulin (11) indicate that most if not all of the endogenous insulin would have disappeared within the two hours after pancreatectomy. The variable results obtained in the four dogs (two showing hypoglycemic effect and two no change) that received the delayed tolbutamide would then be explained by a lack of an adequate amount of recently secreted insulin. This is consistent with the concept that tolbutamide hypoglycemia requires the presence of some insulin.

However, even if tolbutamide hypoglycemia will not occur in the absence of insulin (1), it need not mean that there is a direct relationship or interactivity between them. The two could effect the utilization of glucose at completely different points in the metabolic cycle, but those steps controlled by insulin might have to be intact for tolbutamide to produce hypoglycemia.

These experiments do not exclude the possibility that, in the intact animal, tolbutamide does in addition act by stimulating the pancreas. However, the data presented here do not require that such an explanation be invoked.

SUMMARY

Tolbutamide effects a significant lowering of blood sugar when administered to acutely depancreatized dogs. In these experiments the hypoglycemic action therefore cannot be attributed to a pancreatropic effect. These studies are consistent with the thesis that insulin must be present for tolbutamide to act.

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STUDIES OF THE URICOGENIC EFFECT OF 2-SUBSTITUTED THIADIAZOLES IN MAN *

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Several substances have been found to affect the serum levels of uric acid by influencing the renal mechanisms of uric acid excretion. Among these are agents which inhibit tubular reabsorption of uric acid, *e.g.,* salicylates (1) and probenicid (2), and those which appear to enhance reabsorption, *e.g.,* pyrazinamide (3) and azathymine (4). Other chemical and physical agents are known to produce extensive tissue destruction with resulting elevation of serum and urine uric acid, presumably due in large part to the oxidation of purines coming from the degradation of nucleic acids and soluble nucleotides. This mechanism is most often seen in the leukemias and lymphomas in which X-rays, the nitrogen mustards and adrenocortical steroids can destroy leukocytes and lymphoid tissue with resulting hyperuricemia and uricosuria (5).

In 1956 it was reported (6) that 2-ethylamino-1,3,4 thiadiazole appeared to have the unique property of causing an increase in uric acid production not related to tissue destruction. The purpose of this paper is to describe further experiences with this drug and related compounds. The anti-tumor effects of these agents in animals have been described previously (6–9). Three compounds in this series have been studied in humans: 2-amino-1,3,4 thiadiazole; 2-ethylamino-1,3,4 thiadiazole; and 2,5 diamino-1,3,4 thiadiazole (Figure 1).

MATERIALS AND METHODS

Thirty-three studies were performed in 18 patients with far-advanced, nonresectable cancer (a single patient had hypertensive cardiovascular disease and no known neoplastic disease). All patients were hospitalized during the studies. They received a hospital diet low in purines and an attempt was made to keep the caloric intake constant, although this could not always be rigidly maintained in patients with far-advanced cancer. There were, however, no wide daily fluctuations in dietary intake. Daily 24 hour urine collections were made using thymol as a preservative. If the specimens were not analyzed immediately after collection, aliquots were frozen for storage until analyzed.

Urine uric acid analyses were performed by a colorimetric method employing the enzyme uricase. This method has been described in detail elsewhere (5). It is a modification of the method of Archibald (10) in which color is developed with phosphotungstic acid and sodium polyanethol sulfonate. Duplicate specimens of each sample are analyzed and one specimen of each pair is incubated with uricase [1] prior to color development. The difference between the uricase-treated and the untreated specimens was considered to represent true uric acid. Blood uric acid was measured by the same technique; however, only occasional samples were subjected to incubation with uricase.

This method has been found accurate and highly specific. In order to verify further the authenticity of the material which was measured colorimetrically as uric acid in these studies, specimens of urine from a patient before, during and after treatment with 2-ethylamino thiadiazole were analyzed for uric acid by ultra-

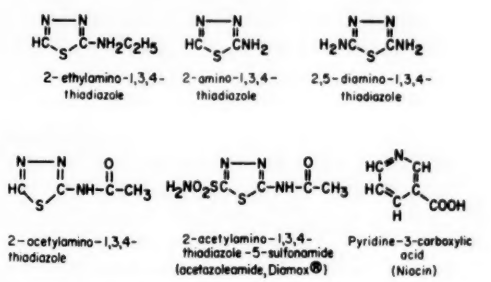

FIG. 1. STRUCTURAL FORMULAE OF THE THIADIAZOLES, NIACIN AND SOME RELATED COMPOUNDS

* This work was supported by Research Grants CY3215, C1889 and CY3190 from the National Cancer Institute, Public Health Service; by a grant from the American Cancer Society and the Damon Runyon Memorial Fund for Cancer Research; and institutional grants from the Lasker Foundation, and the Grant Foundation.

[1] Stero Kems Co., Flushing, N. Y.

violet spectrophotometry (11). The values obtained by this technique correlated closely with those obtained colorimetrically.

Determinations of total nitrogen were made by acid digestion and direct Nesslerization (12).

For determination of isotope content, uric acid was isolated from the urine by precipitating at pH 2. The crude uric acid was redissolved in alkali, decolorized with charcoal and reprecipitated three times. The purity was checked by examination of the ultraviolet absorption spectrum. C^{14} content was determined by assaying known amounts as thin films on aluminum planchets with a Geiger-Muller internal flow counter using helium-isobutane gas. Activity was sufficient to give an accuracy of 10 per cent or better (13). Specimens were prepared for N^{15} analysis as described by Rittenberg (14) by Kjeldahl digestion and treatment of the ammonia with sodium hypobromite. The isotopic ratio was determined with a Consolidated-Nier Model No. 21-201 ratio mass spectrometer.

The thiadiazole compounds[2] are white, water-soluble powders. The formulae are shown in Figure 1. They were administered to patients in single daily doses orally in capsules. The dosage of 2-ethylaminothiadiazole was

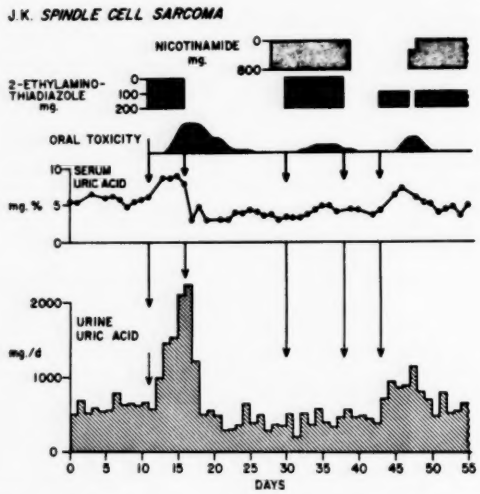

FIG. 2. COURSE OF A PATIENT TREATED WITH 2-ETHYLAMINO-1,3,4 THIADIAZOLE

Note the prompt rise in serum and urine uric acid produced by the thiadiazole alone, the absence of the effect when nicotinamide was given concurrently and the reversal of the effect when nicotinamide was started after the thiadiazole effect had developed.

[2] 2-Amino-1,3,4 thiadiazole and 2-ethylamino-1,3,4 thiadiazole were supplied by Eastman Kodak Co., Rochester, N. Y., and Lederle Laboratories Division of American Cyanamid Co., Pearl River, N. Y. 2,5-Diamino-1,3,4 thiadiazole was purchased from Aldrich Chemical Co., Milwaukee, Wisc.

25 to 200 mg. daily. 2-Amino-thiadiazole was used only at a dose of 50 mg. daily and 2,5-diamino-thiadiazole was given in doses of up to 2 Gm. daily.

Nicotinamide was given orally in divided doses every 6 or 12 hours.

o-Diazo-acetyl-L-serine (azaserine)[3] (15) and 6-diazo-5-oxo-L-norleucine (DON)[3] (16) were given orally in capsules either every eight hours or once daily.

Sodium formate-C^{14}, with an activity of 1 mc. per mM, and adenine-8-C^{14}, with an activity of 8.9 mc. per mM, were given intravenously over a period of 45 minutes. Ammonium chloride-N^{15}, 30 atom per cent excess, was given intravenously in a dose of 0.5 Gm. on each occasion over a period of two hours.

RESULTS

I. 2-Ethylamino-1,3,4 thiadiazole (hereafter called EA-TDA), the first of these compounds to be employed, has been studied in 17 patients.

The results of these studies are shown in Table I. No evidence of tumor regression was seen in any of the patients studied. In each patient, glossitis occurred within four to six days of treatment at a dose of 200 mg. daily. This appeared initially as a diffuse erythema of the tongue, followed by small marginal ulcerations. In the more severe cases, there was ulceration of the gingival surfaces of the lips and erythema of face, perianal region and scrotum or vagina. If smaller doses were used, the onset of glossitis was delayed but occurred when a similar total dose had been given. Regardless of the dose or severity of the reaction, the toxicity subsided within two to five days after administration of EA-TDA was discontinued. In each patient to whom this compound was administered, there was a marked rise in serum uric acid with a parallel rise in urine uric acid. When the administration of the compound was stopped, there was a prompt return to normal in both serum and urine uric acid. The increase in uric acid levels occurred without elevation of the blood urea nitrogen which was measured three to seven times weekly. The urine volumes were adequate and fairly constant during control and treatment periods. Urine total nitrogen was measured in five patients. No significant changes were seen during treatment with EA-TDA, indicating that the uricosuria was not merely representative of a general negative nitrogen balance. An example of the response to this compound is seen in Figure

[3] Supplied by Parke, Davis and Company, Detroit, Mich.

TABLE I

Effect of 2-ethylamino-1,3,4 thiadiazole on uric acid production

Patient, age, diagnosis	Control period		EA-TDA period				EA-TDA and nicotinamide				
	Serum U.A. (max.)	Urine U.A. (mean)	Dose	Days of treatment	Serum U.A. (max.)	Urine U.A. (mean*)	Dose	Nicotinamide	Days of treatment	Serum U.A. (max.)	Urine U.A. (mean)
	mg. %	*mg./day*	*mg./day*		*mg. %*	*mg./day*	*mg./day*	*mg./day*		*mg. %*	*mg./day*
1. J. A., 62 Carcinoma tongue	6.1	296	200	5	24.6	982					
2. S. K., 20 Carcinoma salivary gland	4.0	335	200	3	13.8	759					
3. F. G., 40 Carcinoma breast	4.6	353	200	4	15.0	1,167	200	200	3	12.9	1,134
4. G. R., 64 Carcinoma kidney	7.4	563	200	4	13.2	1,769					
5. H. M., 39 Carcinoma cervix	6.7	227	200	5	11.2	536	200	600	4	6.4	489
6. J K , 34 Fibrosarcoma	6 1	651	200	5	8.9	1,591	200	800	8	4.8	436
7. J. G , 57 Carcinoma rectum	3.0	608	200	4	15.0	1,920					
8. B. C., 56 Hypertensive cardiovascular disease	5.5	667	100	3	15.6	1,599					
6. J. K., 34 Fibrosarcoma	4.4	451	100	5	7.2	920	100	50–800	29	5.0	583
9. B. H., 60 Carcinoma kidney	5.8	489	100	3	11.6	1,093	100	200–400	15	8.3	622
10. J. F., 49 Glioblastoma	2.5	548	100	4	12.4	2,516	100	100	5	3.9	1,479
11. M. M., 41 Carcinoma palate	4.2	446	100	4	10.2	977					
12. A. E., 51 Carcinoma uterus	4.7	671	100	2	15.2	1,122					
13. G. S. Carcinoma lung	4.7	647	100	3	9.9	1,350					
3. F. G., 40 Carcinoma breast	5.2	396	50	15	11.3	901					
3. F. G., 40 Carcinoma breast	5.0	267	25	22	10.5	557	25	200–400	9	9.7	487

* These values indicate the mean uric acid excretion during the period of thiadiazole administration plus one or two days following cessation of administration, the period during which the thiadiazole effect was evident. Control periods were of similar duration—in most cases, an appropriate number of days immediately preceding thiadiazole administration.

2. In addition to the patients listed in Table I, two patients were given the compound without measurement of the urine uric acid. In these, the elevations of the serum uric acid were similar to those seen in the other subjects. Two additional patients were studied in whom renal complications occurred as a result of marked hyperuricemia:

U.F., a 59 year old woman with metastatic melanocarcinoma, was given 100 mg. daily of EA-TDA for four days which resulted in a rise in serum uric acid to 34.8 mg. per cent (control value was 4.5 mg. per cent). She developed oliguria with a rise in blood urea nitrogen (BUN) from 6.6 to 46.5 mg. per cent. The urine output increased after three days of oliguria with a slow fall to normal in serum uric acid and BUN. There was some evidence of renal functional abnormalities for several weeks. Subsequent administration of 25 mg. of EA-TDA daily for two days produced a rise in serum uric acid from 4.6 to 10.3 mg. per cent, with a similar rise in urine uric acid. It was concluded that this patient was unusually sensitive to the effects of this compound on uric acid production.

C.S., a 68 year old man with metastatic anaplastic carcinoma of undertermined origin, received 100 mg. of EA-TDA daily for five days. His serum uric acid rose to 42.4 mg. per cent. Simultaneously, however, he developed nausea and vomiting due to intraperitoneal bleeding. This produced dehydration, oliguria and azotemia. An emergency laparotomy revealed active bleeding from an omental metastasis. He expired five days postoperatively with a markedly elevated serum uric acid and BUN.

TABLE II

Effect of 2-amino-1,3,4 thiadiazole on uric acid production

Patient, age, diagnosis	Control period		A-TDA period			
	Serum U.A. (max.)	Urine U.A. (mean)	Dose	Days of treatment	Serum U.A. (max.)	Urine U.A. (mean)
	mg. %	*mg./day*	*mg./day*		*mg. %*	*mg./day*
9. B. H., 60 Carcinoma kidney	6.3	592	50	4	11.6	929
14. L. A., 70 Carcinoma stomach	4.8	171	50	7	10.0	803
15. S. K., 84 Carcinoma colon	3.8	198	50	5	10.6	392
7. J. G., 57 Carcinoma rectum	3.8	628	50	3	9.4	1,590

II. 2-Amino-1,3,4 thiadiazole (*A-TDA*) has been used in four patients (Table II). The results were similar in every respect to those seen with EA-TDA. The dose-response relationship of this compound has not been explored in detail but the effective dosage appears to be similar to that of EA-TDA.[4]

III. 2,5-Diamino-1,3,4 thiadiazole (*DA-TDA*), the compound of this group which has been found inactive against animal tumors (7-9), has been given in gradually increasing doses up to 2 Gm. daily, 10 times the highest dose of EA-TDA which has been employed (Table III). At this dose, there was no oral toxicity and no effect on uric acid production in patients who had previously shown the capacity to respond to one of the other compounds (Figure 3). One patient was given DA-TDA, 1,500 mg. daily, simultaneously with 100 mg. daily of EA-TDA. The increase in uric acid production was similar to that which occurred when EA-TDA was given alone. The inactive thiadiazole, therefore, did not prevent the "uricogenic" effect of the active one.

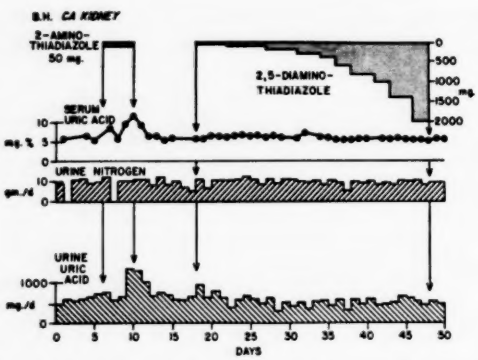

FIG. 3. COURSE OF A PATIENT TREATED WITH 2-AMINO-1,3,4 THIADIAZOLE AND 2,5-DIAMINO-1,3,4 THIADIAZOLE

IV. Reversal studies: A. Nicotinamide. In view of the observations (7-9) that simultaneous administration of nicotinamide could reverse the anti-tumor effect produced by the 2-substituted thiadiazoles in various animal tumors, nicotinamide has been administered in varying doses to patients receiving the thiadiazoles. In the first such patient to be treated (Table I, Patient No. 3), who was previously reported in detail (6), 200 mg. of nicotinamide did not prevent the rise in serum and urine uric acid produced by 200 mg. per day of EA-TDA. However, further experiences with this patient using larger doses of nicotinamide and with all patients treated subsequently revealed that nicotinamide in doses equal to or greater than the dosage of EA-TDA could markedly modify or completely block the effect of the thiadiazole. Furthermore, nicotinamide could reverse the hyperuricemia and glossitis while EA-TDA was continued if these signs were allowed to develop before the institution of nicotinamide therapy. Figure 2 illustrates the observations made in a patient who was given varying doses of EA-TDA with and without nicotinamide. The results of the nicotinamide reversal studies are also shown in Table I. Nicotinamide alone has caused no alteration in uric acid production.

B. DON. Inasmuch as the thiadiazoles have been found to influence purine synthesis, a group of experiments was undertaken in which com-

[4] Since the preparation of this paper 2-acetylamino-1,3,4-thiadiazole has also been found to be uricogenic in a dosage similar to that of EA-TDA.

pounds known to inhibit purine synthesis *in vitro* (17–20) were administered simultaneously with the thiadiazole compounds. In Figure 4 are shown the results of such an experiment in which the glutamine antagonist 6-diazo-5-oxo-L-norleucine (DON) was administered with a dose of EA–TDA which was later shown to be effective in producing a marked rise in serum and urine uric acid in the same patient. It can be seen that when the administration of DON was started prior to the institution of therapy with EA-TDA, the rise in uric acid was appreciably modified. In a similar experiment in another patient, in which EA-TDA was administered with a rise, as expected, in serum and urine acid, subsequent administration of DON while continuing EA-TDA resulted in a return to normal of these values. It is of interest that the characteristic glossitis produced by EA-TDA was not modified by the simultaneous administration of DON. Although DON also can produce glossitis, that which occurred in these cases did not appear to be qualitatively or quantitatively different from that produced by EA-TDA alone.

In an experiment similar to the one shown in Figure 4, *o*-diazo-acetyl-L-serine (azaserine), which is also a glutamine analogue, was given in a dose of 9 mg. per Kg. daily, simultaneously with 200 mg. daily of EA-TDA. It also was effective in nearly completely inhibiting the uricogenic effect of EA-TDA. The appearance of glossitis due to EA-TDA was not prevented.

V. Isotope studies. In order to demonstrate further the uricogenic effect of the thiadiazoles and to help clarify the mechanisms of the effect, the incorporation of labeled purine precursors into uric acid was determined. Figure 5 demonstrates a study in which sodium formate-C^{14} was given

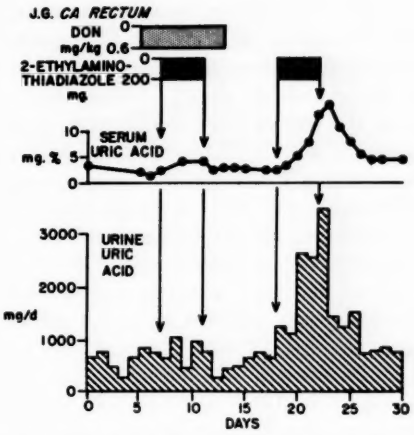

FIG. 4. COURSE OF A PATIENT TREATED WITH 2-ETHYLAMINO-1,3,4 THIADIAZOLE AND 6-DIAZO-5-OXO-L-NORLEUCINE (DON)

Note the marked modification of the thiadiazole effect produced by DON.

intravenously to a patient under three different circumstances. The patient, a 68 year old man with far-advanced, metastatic carcinoma of the lung, was given a purine-free diet throughout the period of observation. As shown in Figure 5 the incorporation of C^{14} into uric acid was markedly increased during treatment with EA-TDA. When EA-TDA and DON were given together the incorporation was similar to that seen during the control period. In a separate study in another patient DON alone was found to produce no effect on the incorporation of sodium formate-C^{14} into uric acid. In that study a maximum activity of 62 cpm per μM was found in a control period with a maximum of 58 cpm per μM during DON administration.

TABLE III

Effect of 2,5-diamino-1,3,4 thiadiazole on uric acid production

Patient, age, diagnosis	Control period		DA-TDA period			
	Serum U.A. (max.)	Urine U.A. (mean)	Dose	Days of treatment	Serum U.A. (max.)	Urine U.A. (mean)
	mg. %	*mg./day*	*mg./day*		*mg. %*	*mg./day*
9. B. H., 60 Carcinoma kidney	6.2	685	50–2,000	30	6.6	514
11. M. M., 41 Carcinoma palate	5.8	333	500–1,500	10	7.4	402

In Figure 6 are shown the results of a study in which the release of uric acid from prelabeled purines was compared with the amount of uric acid synthesized *de novo*. Adenine-8-C^{14} was given to the patient three weeks prior to the administration of EA-TDA. Twenty-four hours after beginning EA-TDA, the patient was given ammonium chloride-N^{15}. A second, identical dose of N^{15}-ammonia was given four weeks following the cessation of administration of EA–TDA. It can be seen that the concentration of uric acid-C^{14} in the urine uric acid fell sharply with the administration of EA-TDA. The marked incorporation of N^{15} into uric acid during and immediately following EA-TDA administration is in contrast to the much smaller incorporation during the control study four weeks later.

DISCUSSION

These compounds have been of interest because they have shown the ability to increase markedly the *de novo* synthesis of uric acid in man. The parallel increase in uric acid content of both serum and urine have ruled out a renal factor as the cause of the hyperuricemia and there have been no effects on other renal functions except when prolonged or excessive hyperuricemia has resulted in renal shutdown.

Tumor breakdown, which can be achieved by a variety of chemical and physical agents and which can sometimes cause definite increases in serum and urine uric acid (5), has been excluded as the cause of the increased uric acid production occurring with the administration of these compounds; aside from the lack of clinical evidence of tumor regression [5] nitrogen excretion has not increased in parallel with the uric acid increase. The uricogenic effect of 2-ethylamino thiadiazole in a patient without a neoplastic disease is further evidence that tumor destruction is not contributing to the uric acid pool.

The final evidence that the increase in uric acid production is due to increased *de novo* synthesis is seen in the isotope studies in which both N^{15}-labeled ammonia and C^{14}-labeled sodium formate were incorporated into uric acid to a much larger extent during thiadiazole administration than during comparable control periods. The sharp drop during EA-TDA administration of the curve of C^{14}-labeled uric acid resulting from the prior administration of adenine-C^{14} indicates a dilution with unlabeled uric acid. Inasmuch as the ribonucleic acid (RNA), desoxyribose nucleic acid (DNA) and acid-soluble purine pool must be assumed to be uniformly labeled, the influx of unlabeled uric acid is considered to represent uric acid newly synthesized from simple precursors.

Although the precise portion of the metabolic pathway in which the thiadiazole effect occurs has not been determined, further evidence that these compounds augment *de novo* biosynthesis of uric acid is seen in the fact that their uricogenic effect is blocked by DON and azaserine, compounds whose major site of action has been localized. These glutamine antagonists have been found to block purine biosynthesis at a relatively simple level, the conversion of formylglycineamide ribo-

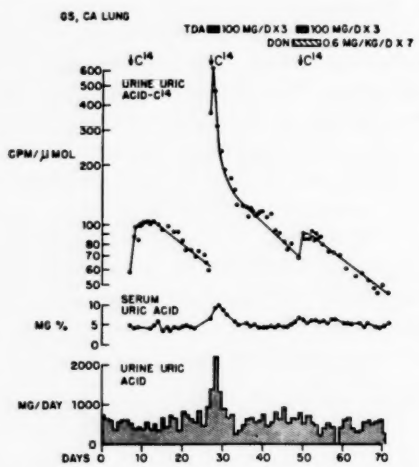

FIG. 5. STUDY OF A PATIENT GIVEN SODIUM FORMATE-C^{14}, 200 μC., ON THREE OCCASIONS

The second and third phases have been corrected here for the extrapolated values of the prior curves since, in these and other studies, the decay curve has been found to be a straight line in a semilogarithmic plot. Note the marked increase in C^{14} concentration in urine uric acid as well as the increase in the amount of urine uric acid produced by 2-ethylamino-1,3,4 thiadiazole and the absence of this effect when DON was given with the thiadiazole.

[5] The inconsistency between the effect of the thiadiazoles on animal tumors and their lack of effect on human tumors is not unusual. Many substances have been found to inhibit the growth of transplanted animal tumors and to be ineffective in spontaneous cancer in man.

nucleotide (FGAR) to formylglycineamidine ribonucleotide (FGAM). They have also been demonstrated (21) to have a marked effect in inhibiting the biosynthesis of uric acid in the intact chicken by blocking the same step in the metabolic pathway. DON and azaserine, however, have not been found in this laboratory to produce a measurable decrease in normal uric acid production in humans although Benedict and co-workers (22) and Wyngaarden (23) have demonstrated that *de novo* synthesis is an important source of uric acid in man. The reversal of the thiadiazole-induced exaggeration of purine (uric acid) synthesis by these known inhibitors of purine synthesis may be only quantitatively different from the failure of DON and azaserine to inhibit normal uric acid synthesis in man. On the other hand, there may be a qualitative difference. The failure of DON and azaserine to prevent the oral toxicity of EA-TDA while inhibiting the uricogenic effect suggests that the action of the thiadiazoles is complex, involving multiple metabolic pathways or involving a single metabolic pathway at multiple levels. It may be postulated that the fundamental mechanism of the thiadiazole effect is in blocking the incorporation of newly synthesized purine molecules into polynucleotides and/or coenzymes with the production of an undefined deficiency state, manifested by glossitis, a sign which is seen in a wide variety of clinical deficiencies. This could stimulate a compensatory exaggeration of purine biosynthesis and the normal oxidation of these purines would result in the observed increase in uric acid production.[6] DON and azaserine may then block the compensatory mechanism without affecting the related deficiency.

Such a deficiency or metabolic block has not been demonstrated. A clue to its nature, however, may be provided by the ability of niacin or nicotinamide to block the toxicity and the uricogenic effects of the thiadiazoles in man and the anti-tumor effects in animals. The reason for the reversal of TDA effects by nicotinamide is not clear. Although the principal physiological function of nicotinamide is in the synthesis of diphosphopyridine nucleotide (DPN), it should be noted that nicotinamide has been found to oppose the activity of a wide variety of chemicals which do not appear to have a common mechanism of action and which have no apparent relationship to DPN synthesis. The system in which this has been most extensively investigated is the developing chick embryo (24, 25), in which a common defect is produced by such unrelated substances as insulin, physostigmine and the nicotinamide analogue, 6-aminonicotinamide, all of which are counteracted by nicotinamide. An identical anatomical lesion is produced by methionine sulfoximine and this cannot be reversed by nicotinamide. Another analogue of nicotinamide, 3-acetylpyridine (26), produces an anatomically different lesion which can also be prevented by nicotinamide. The 2-substituted thiadiazoles produce no specific teratogenic defect in the chick embryo, but their lethality is

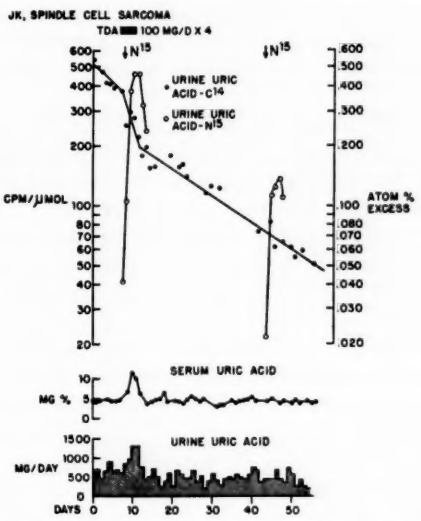

FIG. 6. STUDY OF A PATIENT WHO WAS GIVEN ADENINE-8-C^{14}, 200 μC., THREE WEEKS PRIOR TO 2-ETHYLAMINO-1,3,4 THIADIAZOLE

The thiadiazole was given at a time when the decay curve of urine uric acid-C^{14} was a straight line. Note the sharp drop in C^{14} concentration in urine uric acid produced by the thiadiazole. Ammonium chloride-N^{15} was incorporated to a much greater extent when given during thiadiazole administration than during the control study. The second N^{15} curve has been corrected here for the extrapolated value of the first curve.

[6] Dr. James Wyngaarden has suggested in a personal communication that the thiadiazoles might act by blocking the synthesis of nicotinamide from tryptophan or its binding by desamido-NMN (nicotinamide mononucleotide)-pyrophosphorylase resulting in greater availability of phosphoribosyl pyrophosphate for purine synthesis.

prevented by nicotinamide (27). In this system, as in man, the thiadiazoles cause a marked uricogenic effect (4) which is blocked by nicotinamide.

In the human, 6-aminonicotinamide has been found to produce pellagra-like toxicity characterized by blepharitis, glossitis, tinnitus and deafness, mental confusion and vertigo (28). This toxicity, like that produced by the thiadiazoles, is reversed by nicotinamide. However, at toxic doses of 6-aminonicotinamide, no uricogenic effect has been seen in man (4). It appears that nicotinamide is capable of reversing, in man and in other animal systems, a variety of effects produced by a large, unrelated group of substances. It is clear that there are qualitative differences between the action of these compounds, all reversed by nicotinamide, and these effects cannot be explained by simple competitive antagonism of nicotinamide. If these substances do function as nicotinamide antagonists, they do so by mechanisms that are not precisely the same and they may actually interfere with different functions of nicotinamide.

Their relationship to nicotinamide has suggested that the thiadiazoles produce an alteration in diphosphopyridine nucleotide (DPN), a coenzyme which contains a purine and a nicotinamide moiety. However, such a relationship has not been established. Quantitative alterations in blood DPN levels in man have not been observed following thiadiazole administration.[7] Ciotti, Kaplan, Goldin and Venditti (29) have demonstrated slight substitution of C^{14}-labeled thiadiazole for the nicotinamide moiety of DPN *in vitro* but the significance of this observation has not been determined.

There are other compounds currently in clinical use which are structurally related to the thiadiazoles. Among these are acetazoleamide (Diamox®) which is a 2,5-substituted thiadiazole (Figure 1) and therefore an analogue of 2,5-diaminothiadiazole and some of the bacteriostatic sulfonamides. These have not been found, either in this laboratory or in the reported literature, to produce an increase in uric acid production.

[7] Dr. Ralph Barclay has measured blood levels of DPN in two patients treated with 2-ethylamino-thiadiazole and found no differences from control values in the same patients.

A close parallel has been seen between the uricogenic effects of the thiadiazoles in man and their ability to inhibit growth of a variety of animal tumors. In both of these systems the 2-amino- and 2-ethylamino-thiadiazoles have been uniformly active and the 2,5-diamino-thiadiazole and acetazoleamide uniformly inactive. Since the increase in production of uric acid is clearly not a result of lysis of tumor tissue, the tumor-inhibitory effect cannot be considered the direct cause of the uricogenic effect. However, the parallel suggests that the two apparently diverse effects of this group of compounds are associated with the same or similar metabolic phenomena.

Further studies are in progress to attempt to determine the precise mechanism of action of this group of compounds.

SUMMARY

1. 2-Amino-1,3,4 thiadiazole and 2-ethylamino-1,3,4 thiadiazole have been found to cause an increase in production of uric acid by a mechanism other than nucleic acid degradation. This appears to be due to an increase in uric acid biosynthesis *de novo*.

2. The uricogenic effects of these compounds and their oral toxicity can be blocked or reversed by the administration of nicotinamide in equal or greater doses. The glutamine antagonists, 6-diazo-5-oxo-L-norleucine and *o*-diazo-acetyl-L-serine, block the uricogenic effects but not the oral toxicity.

3. 2,5-Diamino-1,3,4 thiadiazole, a closely related compound, has no effect on uric acid production.

4. The effect or lack of effect of the thiadiazoles on uric acid production in man is closely correlated with their effect or lack of effect in inhibiting growth of animal tumors.

5. The possibility that the effect of these compounds is due to a block in formation of polynucleotides or coenzymes has been suggested.

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A STUDY OF THE MECHANISM BY WHICH EXERCISE INCREASES THE PULMONARY DIFFUSING CAPACITY FOR CARBON MONOXIDE*†

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The pulmonary diffusing capacity (D_L) increases with exercise. This was first shown by Krogh in 1915 (1) and has been confirmed by investigators who have studied the matter since that time, using both carbon monoxide and oxygen (2–5). The mechanism by which exercise increases the diffusing capacity is not altogether clear, however, and the apparent amount of increase with exercise is dependent on the technique used for determining the diffusing capacity, and on the severity of the exercise.

Two methods widely used for estimating the pulmonary diffusing capacity for carbon monoxide are the steady state method of Filley, MacIntosh and Wright (3) and the Krogh breath holding technique as modified by Ogilvie, Forster, Blakemore and Morton (5). In the steady state technique, the subject breathes continuously throughout the test period, which makes minute ventilation a variable factor. In the breath holding method, however, the breath is held and minute ventilation cannot be a variable factor during the actual test period.

The present study was undertaken to investigate the cause of the increased pulmonary diffusing capacity for carbon monoxide which occurs with exercise, using both the steady state and the breath holding techniques. Both methods were used in this study because both have been widely employed for the measurement of diffusing capacity and both show an increase in diffusing capacity with exercise, but the conditions of measurement are different enough to indicate that results with one of these methods might serve as a useful check on the reliability of conclusions reached by the use of the other method. The effects of altering pulmonary blood flow, ventilation, pulmonary vascular pressures, blood pH and blood gas contents, either alone or in combinations, have been studied in an attempt to simulate some of the physiological effects of exercise which might conceivably alter the diffusing capacity. The results indicate that the factors apparently responsible for the increase in pulmonary diffusing capacity with exercise may differ with the technique used.

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METHODS

Subjects. The subjects studied were normal, male, graduate and medical students with an age range of 21 to 26 years and surface area range of 1.70 to 2.14 $M.^2$. All were able to carry out the experimental procedures without difficulty.

Diffusing capacity of the lung for carbon monoxide (D_L). *a) Steady state technique.* Steady state determinations of the pulmonary diffusing capacity were made by the method of Filley and co-workers (3). An indwelling brachial artery needle, and, in those subjects where cardiac output was measured, a superior vena cava catheter, were inserted and the subject was allowed to lie quietly for 30 minutes before the studies were begun. He then inspired 0.1 per cent carbon monoxide in air through a Krogh valve for six minutes and the sample for measurement of D_L was collected during the last two minutes. For determination of D_L during exercise, the subject inspired air for the first two minutes and the 0.1 per cent carbon monoxide mixture for the last four minutes of the six minute period, with sample collection during the last two minutes. Expired gas was collected in a Douglas bag during the fifth and sixth minutes and the number of expirations for this two minute period was recorded. Injection of the T-1824 dye and collection of arterial blood samples at two second intervals for cardiac output determination were carried out as close to the initial part of the two minute collection period as was possible. This usually required 30 to 40 seconds. An arterial blood sample for the determination of pCO_2 was then withdrawn at a slow, regular rate into a heparinized syringe over the two minute period while expired gas was being collected.

Only one exercise determination and a total of no more than three D_L determinations were made in a single subject on any one day. The subject rested at least 20 minutes between each determination of D_L. Control studies in two subjects at rest showed no marked tendency for D_L to decrease progressively as the result of repeated measurements under these conditions. The successive values for D_L obtained with intervening recovery periods of 20 minutes were: for Subject D. G., 27.4, 26.9 and 25.0 ml. CO per minute per mm. Hg; and for Subject J. M., 33.1, 33.8, 33.0 and 32.6 ml. CO per minute per mm. Hg. This result indicated that the change in D_L caused by repeated measurements under these conditions would be small relative to the change produced by exercise. Because the emphasis in this study was on factors which cause a change in the diffusing capacity, rather than on the absolute value of the diffusing capacity, no attempt was made to correct for "back pressure" of carbon monoxide in the pulmonary capillary blood. The order in which D_L under different conditions was measured was randomized from subject to subject in order to minimize systematic error resulting from repeated measurement.

The volume of expired gas was measured in a Tissot gasometer and corrected to the volume at standard temperature and pressure, dry (S. T. P. D.). The expired gas was analyzed, immediately after collection, for carbon monoxide in an infrared analyzer[1] which gave results reproducible to 0.001 per cent carbon monoxide over the concentration range in use (0.000 to 0.100 per cent carbon monoxide). Gas samples for carbon dioxide and oxygen analysis were removed from the Douglas bag immediately after collection and stored over mercury in glass tonometers for analysis within four hours in the micro-Scholander apparatus. Analyses were done in duplicate and required to check within 0.1 per cent. The pH of whole blood was measured with a Cambridge Model R pH meter equipped with an enclosed glass electrode. Measurements were made at room temperature, usually 25 to 26° C., and the result was corrected to 37° C. by Rosenthal's factor (6). The per cent oxygen saturation of arterial blood was assumed to be 97 per cent for calculation of pCO_2 by nomogram. The arterial blood oxygen capacity was estimated from the total hemoglobin, which was determined by the spectrophotometric method of Drabkin and Austin (7). The carbon dioxide content of whole blood was determined by the method of Van Slyke and Neill (8), and duplicate analyses were required to check within 0.1 volume per cent. The plasma carbon dioxide content was estimated from the blood carbon dioxide content and from the pH, hemoglobin concentration and per cent oxygen saturation by the line charts of Van Slyke and Sendroy (9). The plasma CO_2 tension was calculated by the Henderson-Hasselbalch equation, using a pK′ of 6.11 (10). In this laboratory, pCO_2 values by this method are reproducible to within ±1.0 mm. Hg.

Calculations of D_L were made as outlined by Filley and co-workers (3):

$$\dot{V}_{CO} = \dot{V}_E \left(F_{ICO} \frac{F_{E N_2}}{F_{I N_2}} - F_{ECO} \right)$$

[1] Model 15A, Liston Becker Co.

and

$$P_{ACO} = (Bp - 47) \frac{F_{ECO} - r F_{ICO}}{1 - r}$$

where

$$r = \frac{Vd' \text{ (physiological dead space volume)}}{Vt \text{ (tidal volume)}} = \frac{P_{ACO_2} - P_{ECO_2}}{P_{ACO_2}}$$

$$D_L = \frac{\dot{V}_{CO}}{P_{ACO}}.$$

$\dot{V}_{CO}$ is the minute uptake of CO, $\dot{V}_E$ is the total gas volume expired per minute, F_I and F_E are the inspired and expired concentrations of the gases indicated by subscripts, P_{ACO} is the alveolar tension of CO, and P_{ACO_2} and P_{ECO_2} are arterial and expired tensions of CO_2, respectively.

When the inspired mixture contained 5.0 per cent CO_2,

$$r = \frac{P_{ACO_2} - P_{ECO_2}}{P_{ACO_2} - P_{ICO_2}}.$$

Since D_L is expressed as the quantity of CO diffused per minute per mm. Hg CO pressure gradient between alveolar gas and capillary blood, the correct equation for D_L is $\frac{\dot{V}_{CO}}{P_{ACO} - P_{CCO}}$, where P_{CCO} represents the mean carbon monoxide tension of pulmonary capillary blood. Filley and co-workers (3) regarded P_{CCO} as negligible and considered the pressure gradient substantially equal to the alveolar pressure of CO, thus omitting P_{CCO} from the equation for expediency. In the present study, P_{CCO} has been omitted from the equation and no correction for back pressure of CO was made in the calculations. This is not entirely correct for measurement of the absolute value of D_L, since it has been shown that P_{CCO} does effect steady state determinations (11, 12), and repeated steady state measurements might be expected to show progressive decline in apparent D_L because of the progressive accumulation of COHb. As pointed out previously, however, repeated measurements of D_L at intervals of 20 minutes in control subjects in the resting state indicated that the D_L was not being significantly altered, for the purposes of this study, by the changes in pulmonary capillary tension of CO brought about by preceding measurements.

Hyperventilation is one of the obvious consequences of exercise, and the effect on steady state D_L of hyperventilation without other exercise was therefore examined. The procedure for measurement of D_L by the steady state technique during hyperventilation requires description in some detail because of uncertainties which hyperventilation introduces into the method. The principal uncertainties are as follows:

1. The steady state method embodies the assumption that the physiological dead space and intrapulmonary distribution of carbon monoxide and carbon dioxide are similar, but it is uncertain how this relationship is affected by hyperventilation. However, the carbon monoxide dead space increases by about the same amount as that for carbon dioxide during the hyperpnea of exercise in normal persons (4), and it appears to be a reasonable assumption that this relationship also holds during voluntary hyperventilation.

2. Hyperventilation also imposes significant technical difficulties: *a*) As the difference between $P_{A_{CO_2}}$ and $P_{E_{CO_2}}$ is progressively decreased by hyperventilation on air or, especially, on 5.0 per cent CO_2, an error in the determination of either $P_{A_{CO_2}}$ or $P_{E_{CO_2}}$ will cause a progressively greater error in the determination of "r" and, consequently, in D_L. *b*) Failure to achieve a steady state may result in sampling which is unrepresentative of the entire test period with consequent possibility of large errors. In particular, overestimating mean $P_{A_{CO_2}}$ by about 4 mm. Hg during hyperventilation on air could cause an apparent increase in D_L equal to that reported in Table V as resulting from hyperventilation. Underestimating $P_{A_{CO_2}}$ causes D_L to be underestimated, but the absolute error is less than that caused by overestimation of $P_{A_{CO_2}}$. *c*) Failure to reach a steady state can cause some error even though ventilation rate and cardiac output are constant and arterial blood is sampled at a constant rate throughout the period of collecting expired air. This is because the CO_2 dissociation curve is not a straight line, so that the pooled arterial sample, although it represents accurately the mean CO_2 content of blood when this is changing during the collection period, may not represent exactly the mean CO_2 tension. Sample calculations indicate, however, that the error on this account is negligible for present purposes.

Because of these uncertainties, care was taken to keep hyperventilation as regular as possible. The frequency of respiration was timed with a metronome at 22 breaths per minute and the subjects were asked to keep tidal volume as constant as possible. Hyperventilation was maintained on the carbon monoxide mixture for six minutes, during the last two minutes of which expired gas was collected and arterial blood was continuously drawn at a steady rate for the determination of D_L. This procedure was carried out for hyperventilation on 0.1 per cent CO in air as well as 0.1 per cent CO in a mixture with 5.0 per cent CO_2. During these few minutes a condition of complete equilibrium was certainly not attained. For the six subjects of Table V, the control respiratory exchange ratio was 0.96 (S.D., 0.13) and that during hyperventilation on air was 1.33 (S.D., 0.20). For the three subjects who also hyperventilated on 5.0 per cent CO_2, the values at this time were 0.36, 0.51 and 0.67. The question of importance for the present use of the steady state method, however, is the extent to which conditions were stable during the test period. To examine this question, end-expiratory alveolar air samples were collected from the first three subjects at the end of each test period and the $P_{A_{CO_2}}$ calculated from these samples was compared with the mean $P_{A_{CO_2}}$ of the arterial blood sample which was drawn continuously through the two minutes of the test period. For the resting state, the values of arterial minus alveolar pCO_2 were -0.1, 0.9 and -0.6 mm. Hg; for hyperventilation on air, they were 1.9, -0.1 and 1.2 mm. Hg; and for hyperventilation on CO_2, they were -0.2, -0.2 and 0.2 mm. Hg. These results indicate that conditions during the test period were adequately steady for use of the method. As an additional check, the oxygen contents of inspired, expired and alveolar air were used for an independent calculation of "r" and D_L. This precaution is especially desirable in experiments dealing with hyperventilation on CO_2, since analytical errors in CO_2 measurements here can cause large errors in estimation of D_L. As an auxiliary test gas, oxygen also has the advantages of being somewhat less sensitive than CO_2 to variations in the steady state, and of having physical properties close enough to carbon monoxide to make it probable that intrapulmonary distribution of the two gases during hyperventilation is similar. Results with the two test gases were quite similar, as shown in Table V, which presents D_L values obtained during the same experiments using arterial pCO_2, alveolar pCO_2 and alveolar pO_2. These all show substantial agreement.

These experiments indicate that the steady state method can be used satisfactorily during hyperventilation under the conditions of this investigation.

b) *Breath holding technique.* Measurements of the breath holding D_L were made by the modification of the Krogh technique described by Forster and co-workers (5, 13). Using this technique, the subject made a maximal inspiration from the level of residual volume, held his breath for approximately 10 seconds with the glottis open, avoiding a valsalva maneuver, and then rapidly expired, delivering the entire expirate within two seconds. All of the expired air except the first liter, usually amounting to between 2.5 and 3 L., was collected to be analyzed as alveolar gas. The inspired gas mixture contained 0.3 per cent carbon monoxide, 10 per cent helium and approximately 20 per cent oxygen in nitrogen and was inspired from a bag in a box connected with a Collins spirometer so that the inspired volume was recorded on the spirometer. The exact duration of breath holding, timed from the beginning of inspiration to the start of collection of the alveolar sample, was recorded on the spirometer kymograph drum, which was turning at a constant rate. The alveolar sample was collected through a stopcock in the circuit near the subject's mouth. After the subject expired approximately 1 L. (as observed on the spirometer tracing), which was considered more than adequate to wash out the dead space, the stopcock was turned so that the remainder of the expirate was diverted into a collapsible plastic bag, previously evacuated to low pressure by a pump. Residual volume was determined in each subject by the open circuit helium method of Hickam, Blair and Frayser (14) and, since inspiration was made from residual volume, alveolar volume was taken as the sum of residual volume and inspired volume.

The alveolar gas samples were analyzed for helium, carbon monoxide and oxygen. The helium concentration was determined by katharometer[2] with an accuracy of about one part in 100 or 0.05 per cent helium (14). Carbon monoxide concentration was measured by the infrared analyzer with an accuracy of about one part in 100 or 0.001 per cent carbon monoxide. Analyses for helium and carbon monoxide were made after removal of CO_2 by passage through "ascarite." The helium and carbon monoxide concentrations obtained in this manner were then corrected for the volume change resulting from removal of carbon dioxide prior to analysis. In order to make this correction, determination of carbon dioxide con-

[2] Hastings Instrument Co.

centration in the alveolar sample was required. This was done indirectly as follows. The oxygen concentration in the dried gas sample was determined by a Pauling analyzer,[3] both before and after removal of carbon dioxide by passage through "ascarite." The carbon dioxide concentration could then be estimated by the change in oxygen content of the gas sample resulting from carbon dioxide absorption. Change in oxygen concentration can be measured, under these conditions, with an accuracy of 0.1 per cent. Estimate of carbon dioxide by this means is relatively crude, but is accurate to less than 0.5 per cent. This degree of inexactitude introduces an error of less than one part in 200 in the estimate of carbon monoxide and helium concentrations. In the helium analysis, allowance was made for the effect on the katharometer of changes in the oxygen-nitrogen ratio in the expired gas from the value at which the katharometer was calibrated (0.26). A decrease in the ratio caused an apparent decrease in helium concentration as measured by the katharometer, amounting to 0.17 per cent helium for a change in ratio from 0.3 to 0.2. The appropriate correction was made in each case.

For the studies in which the cardiac output was measured, the indwelling brachial artery needle and superior vena cava catheter were put in place at least 30 minutes before the tests were begun. At least 10 minutes were allowed between each determination of D_L by the breath holding method.

The carbon monoxide concentration present in the alveolar sample before any had been absorbed by the lungs was calculated from the dilution of helium by the equation of Forster and co-workers (5, 13):

$$\text{Initial CO concentration in the expired alveolar sample} = \frac{\text{He concentration in the expired alveolar sample}}{\text{Inspired He concentration}} \times \text{Inspired CO concentration.}$$

D_L was then calculated from Krogh's equation (1):

$$D_L = \frac{\text{Alveolar volume (S.T.P.D.)} \times 60}{\text{Time (seconds)} \times (Bp - 47)} \times \text{Natural log} \frac{\text{Initial CO concentration in the expired alveolar sample}}{\text{Final CO concentration in the expired alveolar sample}}$$

with D_L expressed as ml. CO (S.T.P.D.) diffused per minute per mm. Hg CO tension.

Measurement of cardiac output and central venous pressure. Cardiac output was determined by the dye dilution technique (15), with central venous injection of the dye as described by Doyle, Wilson, Lepine and Warren (16), using T-1824. A No. 6 calibrated catheter was inserted through an antecubital vein and the end placed in the superior vena cava by fluoroscopic observation so that T-1824, in 0.5 per cent solution, could be injected from a calibrated syringe into the central circulation. After the dye was injected, arterial blood samples were collected in 3 ml. siliconized tubes at two second intervals. The undiluted dyed serum from each two second sample was read on a Beckman DU spectrophotometer against a serum blank. The optical densities were plotted against time on semilogarithmic graph paper and all the points from the first appearance of the dye in the serum until recirculation began were used in construction of a curve. With exercise and with the cardiac output increased by drugs, from five to eight points were available for constructing the curve. Under all other conditions, from seven to 11 points were used. Calculations of the cardiac outputs were then made from the time-concentration curves (16).

Since the purpose of cardiac output measurements was to study the relationship of cardiac output to D_L, it would have been desirable to make the measurements of cardiac output at precisely the same time D_L was being determined. This, however, would have required a second arterial puncture for the steady state procedure since an arterial blood sample for pCO_2 determination was being withdrawn continuously throughout the two minute D_L determination period. Dye injections and arterial sample collections for cardiac output determinations, therefore, were carried out immediately before the arterial blood for pCO_2 was taken.

[3] Model E-2, Arnold O. Beckman Inc.

When D_L was determined by the breath holding technique, it was again technically not feasible to collect the arterial blood samples for cardiac output measurement at the precise time D_L was being measured. For this reason, the procedures for measuring cardiac output were carried out in the 30 to 40 seconds immediately before D_L determination. In order to maintain conditions comparable to those during the measurement of D_L, the breath was also held with glottis open during the injection of dye and the collection of arterial blood samples. The same technique was used each time a cardiac output was measured together with a breath holding D_L under various experimental conditions.

The central venous pressure was measured by way of the superior vena cava catheter, using a Statham strain gage (0 to 25 mm. Hg), and was recorded by a direct writing recorder. The point of zero reference was arbitrarily taken at a position midway between the table surface and the fourth costochondral junction. The matter of primary interest was change in pressure rather than absolute value of pressure.

Exercise. Exercise was carried out while supine by having the subjects alternately flex and extend the legs rapidly, sliding the heels along the table top. The subjects exercised for six minutes for both steady state and breath holding studies.

Procedures for increasing cardiac output without exercise. Cardiac output was increased by drugs in six subjects studied by the steady state method and in 12 subjects studied by the breath holding technique. In three subjects of the steady state group, the cardiac output was increased by epinephrine, 0.17 to 0.19 μg. per Kg. per minute, given by intravenous infusion over a 10 minute period before the D_L and cardiac output were determined and maintained during these determinations. In the other

TABLE I

Steady state D_L, cardiac output and ventilation rate, in 19 normal supine subjects at rest

Subject	Age	Surface area	Number of determinations	Ventilation rate*	Cardiac output	D_L
		M.²		*L./min.*	*L./min.*	*ml./min./mm. Hg*
D. P.	22	1.86	1	8.1	7.8	24.4
R. D.	25	1.96	2	7.4	6.1	32.4
				6.5	6.8	23.5
F. D.	24	2.10	2	6.3	7.5	24.6
				6.7	9.3	23.5
R. R.	24	2.14	1	8.8	6.7	21.9
S. S.	22	1.70	2	7.8	6.4	25.1
				5.3	4.2	19.6
C. R.	20	1.82	2	4.7	6.7	23.9
				6.1	9.4	21.7
H. S.	22	1.72	2	5.8	10.0	22.6
				7.4	6.4	22.0
S. W.	23	1.97	1	4.7	6.1	27.2
D. G.	22	2.06	1	6.5	7.1	19.9
C. W.	24	1.87	1	5.0	5.4	24.5
B. S.	21	1.88	1	5.4	6.2	24.6
R. R.	26	1.75	1	6.8	6.7	19.9
S. R.	22	1.70	1	7.4		22.0
B. C.	23	2.00	1	7.6	6.1	24.3
B. M.	22	1.96	1	4.9	5.8	21.3
J. F.	23	1.86	1	5.8	6.0	19.4
J. S.	21	2.08	1	8.7	10.3	29.2
F. A.	21	1.76	1	7.6	7.7	22.9
J. B.	21	2.12	1	7.1	9.4	23.6
Mean	22.5	1.91		6.6±1.2†	7.0±1.4	23.5±3.0

* All ventilation rates are in L. per minute S.T.P.D.
† Values following the means are standard deviations.

three subjects of the steady state group, and in all the 12 subjects where breath holding D_L was determined while the cardiac output was increased by drugs, the cardiac output was increased by a combination of atropine sulfate and norepinephrine, which has also been found effective in increasing cardiac output by Bryant, Combs and Warren (17). Atropine sulfate (2.0 mg.) was given intravenously over a two minute period followed by an intravenous infusion of norepinephrine given at the rate of 8.0 μg. per minute over a six minute period before the determinations of D_L and cardiac output were made and maintained during these determinations.

In some experiments, the cardiac output was increased by producing reactive hyperemia in the legs with arterial tourniquets (18). The tourniquets were 6 inch sphygmomanometer cuffs which were placed around the upper thighs, inflated, and maintained at a pressure of 200 mm. Hg for 15 minutes. The pressure was relieved suddenly by an escape valve. This procedure, which acutely reduces peripheral resistance, will approximately double the resting cardiac output for about a minute.

Procedures intended to produce pulmonary vascular engorgement. The effect of procedures designed to increase the pressure in the pulmonary capillary bed on the steady state D_L was studied in several subjects. The procedures used were rapid expansion of the plasma volume by intravenous infusion of human serum albumin solution and reduction in size of the peripheral vascular bed by norepinephrine infusion or by compression of the body by an aviator's "G suit."[4] Pulmonary vascular pressures were not measured. It has been found, however, that these procedures will increase central venous pressure in normal subjects by about the same amount that they increase pulmonary capillary and pulmonary artery pressures. Changes in central venous pressure (CVP) were therefore used as a rough index of change in pulmonary vascular pressures. The support for this concept is provided by the work of several different investigators. Doyle, Wilson, Estes and Warren found that pulmonary arterial and capillary pressures were increased by rapid infusion of saline solution (19) to about the same extent that such infusions increase central venous pressure (20). They concluded that the rise in pressure produced in this way is essentially the same throughout the central veins and pulmonary vessels, with maintenance of normal pressure gradient at a higher level. Fowler, Westcott, Scott and McGuire (21) found that norepinephrine infusion produced a rise in pulmonary capillary pressure, and, in the three subjects where it was measured, a similar increase in right atrial pressure. Wood, Lewis, Forster and Beckman (22, 23) found that inflation of a "full pressure half-suit" around the lower half of the body produced similar increases in central venous, right heart, pulmonary artery and pulmonary capillary pressures.

In the present study, four subjects had an infusion of between 400 and 500 ml. of 5.0 per cent serum albumin in physiological saline over a six minute period to produce

[4] Made by the David Clark Co., Inc., Worchester, Mass.

pulmonary vascular engorgement while the steady state D_L was being determined.

In three subjects, inflation of the G suit over the lower half of the body was used alone to produce pulmonary vascular engorgement. An attempt was made to keep the suit inflated at high pressure (two pounds per square inch) to cause marked increase in the CVP for the entire six minute period of the steady state D_L measurement. This was uncomfortable, however, and therefore the pressure during this time was alternately applied and released at 15 second intervals.

Pulmonary vascular engorgement was produced in two subjects by a combination of norepinephrine infusion with G suit inflation. When the suit was inflated to 1.5 pounds per square inch, it was found that the CVP was at first elevated but then decreased gradually even though G suit pressure was maintained. Bondurant, Hickam and Isley (24) observed this previously and used norepinephrine infusion to maintain the increase in CVP produced by G suit inflation. For this reason, norepinephrine infusion was added to G suit inflation in these two subjects, and the CVP remained elevated throughout the six minute period.

In six subjects, a combination of G suit inflation (1 to 1.5 pounds per square inch) and infusion of 300 to 400 ml. of 5.0 per cent serum albumin solution in saline over the six minute D_L determination period was used to cause pulmonary vascular engorgement. With this method, high central venous pressures were produced and maintained for the six minute period without causing significant discomfort.

RESULTS

A. *Studies with D_L determined by the steady state method*

The studies in normal resting subjects are summarized in Tables I and II. Nineteen supine subjects had a mean D_L of 23.5 (SD ±3.0) ml. per minute per mm. Hg. Six other subjects in the seated position had a mean D_L of 25.5 ± 5.3 ml. per minute per mm. Hg. In this group of subjects, the differences between seated and supine D_L values are not significant.

TABLE II

Steady state D_L, ventilation and cardiac output in six normal seated subjects at rest

Subject	Age	Surface area	Ventilation rate	Cardiac output	D_L
		M.²	*L./min.*	*L./min.*	*ml./min./mm. Hg*
D. J.	21	1.84	6.8	5.3	22.0
F. D.	24	2.14	5.8	7.2	23.5
C. H.	21	2.14	7.8	4.5	26.7
R. C.	23	2.02	7.9	8.4	35.8
B. L.	23	2.14	8.3	7.6	23.1
J. C.	23	1.79	6.5	9.5	22.0
Mean	22.5	2.01	7.2±0.9	7.1±1.9	25.5±5.3

Effect of exercise and increased cardiac output on the steady state D_L

Cardiac output and D_L were measured simultaneously in six subjects, first at rest, then during exercise, and finally during a period when the cardiac output was increased by drugs while the subjects were otherwise at rest, as summarized in Table III (see Methods). With exercise, the mean cardiac output increased to 13.6 ± 3.9 L. per minute from the resting value of 7.7 ± 1.6 L. per minute, while the mean D_L increased to 39.8 ± 5.5 ml. per minute per mm. Hg from the resting mean of 25.4 ± 3.6 ml. per minute per mm. Hg. When the cardiac output was comparably increased by drugs without change in the mean ventilation rate, there was no increase in D_L over the resting level.

In four subjects, the intravenous infusion of 400 to 500 ml. of 5.0 per cent human serum albumin in normal saline increased the mean cardiac output to 10.7 ± 1.2 L. per minute from 6.7 ± 2.6 L. per minute at rest before the infusion, but the mean D_L remained substantially unchanged (Table IV).

In summary, exercise caused a striking increase in steady state D_L, but increasing the cardiac output to the same extent by drugs or albumin infusion did not change D_L.

Effect of increased ventilation rate on the steady state D_L

The effect of voluntary hyperventilation, carried out as previously described, on the steady state D_L was studied in six subjects who were otherwise at rest (Table V). The mean ventilation rate changed from 6.1 ± 0.9 to 16.0 ± 3.6 L. per minute, while the mean cardiac output remained essentially the same as at rest. The mean D_L increased from the resting level of 22.3 ± 2.2 ml. per minute per mm. Hg to 38.3 ± 7.1 ml. per minute per mm. Hg with hyperventilation.

The first three subjects shown in Table V hyperventilated while breathing 0.1 per cent carbon monoxide in a mixture with 5.0 per cent carbon dioxide as well as while breathing 0.1 per cent carbon monoxide in air. Hyperventilation while breathing the carbon dioxide mixture prevented the arterial pH and pCO_2 changes which occurred with hyperventilation on air, but the

TABLE III

Steady state D_L, ventilation rate, cardiac output and oxygen uptake, in six supine subjects at rest, during exercise, and with the cardiac output increased by drugs

Subject	Condition	M.V.*	O_2 uptake	C.O.†	D_L
		L./min.	*ml./min.*	*L./min.*	*ml./min./mm. Hg*
R. D.	Rest	7.4	320	6.1	32.4
	Exercise	28.7	1,350	19.7	37.0
	Epinephrine	6.7	324	12.1	31.2
F. D.	Rest	6.3	295	7.5	24.6
	Exercise	20.1	892	14.3	47.9
	Epinephrine	6.0	231	14.8	23.3
S. S.	Rest	7.8	306	6.4	25.1
	Exercise	17.4	669	9.8	34.9
	Atropine and norepinephrine	7.5	342	10.8	20.7
C. R.	Rest	4.7	220	6.7	23.9
	Exercise	13.9	634	10.2	36.5
	Atropine and norepinephrine	6.5	302	13.1	28.1
H. S.	Rest	5.8	263	10.0	22.6
	Exercise	14.2	659	11.3	36.8
	Atropine and norepinephrine	7.1	296	14.2	20.5
F. D.	Rest	6.7	290	9.3	23.5
	Exercise	17.4	696	16.1	45.4
	Atropine and norepinephrine	6.6	288	16.4	24.0
Means	Rest	6.5 ± 1.1	282 ± 35.9	7.7 ± 1.6	25.4 ± 3.6
	Exercise	18.6 ± 5.5	817 ± 277.0	13.6 ± 3.9	39.8 ± 5.5
	Drugs	6.7 ± 0.5	297 ± 38.0	13.6 ± 2.0	24.6 ± 4.2

* Minute ventilation.
† Cardiac output.

TABLE IV

Effect of procedures designed to increase pulmonary vascular pressures on the steady state D_L in six supine subjects

Subject	Condition	M.V.	C.O.	C.V.P.*	D_L
		L./min.	*L./min.*	*mm. Hg*	*ml./min./mm. Hg*
S. S.	Rest	5.3	4.2	3.5	19.6
	Albumin infusion	6.8	9.6	9.6	22.1
	G suit and albumin infusion	4.8	5.8	17.0	19.0
H. S.	Rest	7.4	6.4	−0.3	22.0
	Albumin infusion	6.3	11.7	3.3	23.0
	G suit and albumin infusion	5.4	7.2	8.8	26.0
J. F.	Rest	5.8	6.0	0.5	19.4
	Albumin infusion	4.6	9.7	4.5	21.3
	G suit and albumin infusion	4.8	6.1	16.2	20.2
J. S.	Rest	8.7	10.3	0.5	29.2
	Albumin infusion	8.3	11.6	3.2	29.4
	G suit and albumin infusion	7.2	12.7	7.0	33.4
F. A.	Rest	7.6	7.7	0.9	22.9
	G suit and albumin infusion	7.2	6.3	12.9	21.2
J. B.	Rest	7.1	9.4	8.9	23.6
	G suit and albumin infusion	8.9	10.3	23.0	31.3
Means	Rest (first 4 subjects)	6.8 ± 1.6	6.7 ± 2.6	1.1 ± 1.7	22.6 ± 4.6
	Albumin infusion	6.5 ± 1.5	10.7 ± 1.2	5.2 ± 3.0	24.0 ± 3.7
	Rest (all 6 subjects)	7.0 ± 1.2	7.3 ± 2.3	2.3 ± 3.5	22.8 ± 3.6
	G suit and albumin infusion	6.4 ± 1.6	8.1 ± 2.8	14.2 ± 5.9	25.2 ± 6.1

* Central venous pressure.

TABLE V

Effect of changes in ventilation rate on the steady state D_L in six supine subjects

Subject	Condition	M.V.	C.O.	pCO_2*	(1)† D_L	(2)† D_L	(3)† D_L
		L./min.	*L./min.*	*mm. Hg*	*ml./min./mm. Hg*		
D. G.	Rest	6.5	7.1	38.4	19.9	20.0	19.9
	Air hypervent.	20.8	8.1	24.0	45.7	38.7	49.2
	CO_2 hypervent.	22.5	8.5	42.7	43.2	45.3	42.9
C. W.	Rest	5.0	5.4	38.8	24.5	21.9	22.0
	Air hypervent.	11.7	5.8	27.6	29.1	29.3	29.4
	CO_2 hypervent.	19.5	8.6	46.5	29.8	30.6	29.5
B. S.	Rest	5.4	6.2	40.5	24.6	26.1	25.6
	Air hypervent.	13.9	6.4	29.4	41.4	36.9	43.3
	CO_2 hypervent.	17.8	7.0	46.8	37.1	36.0	39.5
R. R.	Rest	6.8	6.7	41.8	19.9		
	Air hypervent.	12.1	7.3	28.9	27.9		
C. R.	Rest	6.1	9.4	39.3	21.7		
	Air hypervent.	19.4	9.5	24.6	41.2		
R. D.	Rest	6.5	6.8	42.4	23.5		
	Air hypervent.	18.3	6.9	28.6	44.5		
Means	*a) First 3 subjects*						
	Rest	5.6	6.2	39.2	23.0	22.7	22.5
	Air hypervent.	15.5	6.8	27.0	38.7	35.0	40.6
	CO_2 hypervent.	19.9	8.0	45.3	36.7	37.3	37.3
	b) All 6 subjects						
	Rest	6.1 ± 0.9	6.9 ± 1.2	40.2 ± 1.5	22.3 ± 2.2		
	Air hypervent.	16.0 ± 3.6	7.3 ± 1.2	27.2 ± 2.1	38.3 ± 7.1		

* Arterial blood.

† D_L (1) is calculated using arterial blood pCO_2 to determine "r," D_L (2) is calculated using alveolar pCO_2 to determine "r," and D_L (3) is calculated using alveolar pO_2 to determine "r."

effect on D_L was the same. For the reasons already discussed (see Methods), D_L in these three subjects was calculated with "r" determined using arterial blood pCO_2, with "r" determined using alveolar pCO_2, and with "r" determined using alveolar pO_2. The values for D_L calculated by the three different methods are in close agreement.

In summary, voluntary hyperventilation, whether on air or 5.0 per cent CO_2, caused the same apparent increase in steady state D_L as occurred with exercise which induced an equal rise in ventilation rate.

Effect on the steady state D_L of procedures designed to increase pulmonary vascular pressures

A study was made of the effect on steady state D_L of procedures designed to increase pulmonary vascular pressures by pulmonary vascular engorgement. The four subjects who received an intravenous infusion of 5.0 per cent human serum albumin in normal saline (Table IV) had a mean rise in central venous pressure of 4.1 mm. Hg, a concomitant rise in mean cardiac output to 10.7 ± 1.2 L. per minute from 6.7 ± 2.6 L. per minute at rest, and no change in mean ventilation rate. The mean D_L was 22.6 ± 4.6 ml. per minute per mm. Hg in the control period and 24.0 ± 3.7 ml. per minute per mm. Hg with the albumin infusion. In six subjects, inflation of a G suit, in combination with serum albumin infusion, produced a mean increase in central venous pressure of 11.9 mm. Hg without significant change in mean ventilation rate or cardiac output. The mean D_L was 22.8 ± 3.6 ml. per minute per mm. Hg at rest and 25.2 ± 6.1 ml. per minute per mm. Hg with the increased central venous pressure (Table IV). When these 10 instances in which pulmonary vascular engorgement was produced by either albumin infusion or G suit inflation with albumin infusion are considered together, a small but statistically significant increase in D_L, amounting to 2.0 ml. per minute per mm. Hg ($p < .05$) (25), was

TABLE VI

Effect of procedures designed to increase pulmonary vascular pressures on the steady state D_L in three supine subjects

Subject	Condition	M.V.	C.O.	C.V.P.	D_L
		L./min.	*L./min.*	*mm. Hg*	*ml./min./mm. Hg*
B. C.	Rest	7.6	6.1	1.0	24.3
	G suit*	20.8	8.1	up 3.3	43.3
				down 0.7	
	G suit and norepinephrine infusion	14.8	7.2	5.9	29.2
B. M.	Rest	4.9	5.8	−0.4	21.3
	G suit*	8.1	4.2	up 7.3	28.8
				down 1.7	
	G suit and norepinephrine infusion	13.1	5.5	8.8	40.8
J. B.	Rest	7.1	9.4	8.9	23.6
	G suit*	9.4	9.1	up 26.1	28.0
				down 8.9	
Mean	Rest (first 2 subjects)	6.3	6.0	0.3	22.8
	G suit and norepinephrine infusion	14.0	6.4	7.4	35.0
	Rest (3 subjects)	6.5	7.1	3.2	23.1
	G suit*	12.8	7.1	up 12.2	33.4
				down 3.8	

* With alternate application and release of pressure as described in Methods.

associated with the pressure elevation (Table IV). This increase in D_L was not dependent upon an increase in ventilation rate, since ventilation was actually lower in most of the subjects during the period of pulmonary vascular engorgement than during the control period.

In two subjects who had a combination of G suit inflation and norepinephrine infusion, mean CVP was increased by 7.1 mm. Hg without change in the cardiac output (Table VI). The mean ventilation rate, however, also increased. Mean D_L in these subjects increased from 22.8 ml. per minute per mm. Hg at rest to 35.0 ml. per minute per mm. Hg.

The three subjects who were studied during G suit inflation in which the pressure was alternately increased and decreased, as already described, had a significant rise in central venous pressure, but this was also accompanied by an increase in mean ventilation rate. D_L increased from 23.1 ml. per minute per mm. Hg at rest to 33.4 ml. per minute per mm. Hg (Table VI).

In summary, various procedures designed to increase pulmonary capillary pressure by engorging the lungs with blood produced only minor changes in steady state D_L unless they also caused an increase in minute ventilation; D_L showed a substantial increase only when ventilation was increased.

Effect of a combination of albumin infusion with increased ventilation on the steady state D_L

To investigate the possibility that increased pulmonary vascular pressures could enhance the effect of hyperventilation on D_L, three subjects were studied, first at rest, then with hyperventilation, and lastly with hyperventilation during an intravenous infusion of 400 to 500 ml. of 5.0 per cent human serum albumin in normal saline (Table VII). Hyperventilation alone, with a mean ventilation rate of 16.6 L. per minute, increased mean D_L to 37.9 ml. per minute per mm. Hg from 21.7 ml. per minute per mm. Hg at rest. About the same mean ventilation rate, 16.3 L. per minute, accompanied by a 6.8 mm. Hg increase in the central venous pressure produced by the albumin infusion, increased the

TABLE VII

Effect of a combination of albumin infusion with an increased ventilation rate on the steady state D_L in three supine subjects

Condition	M.V.*	C.O.*	C.V.P.*	D_L
	L./min.	*L./min.*	*mm. Hg*	*ml./min./mm. Hg*
Rest	6.5	7.6	−0.5	21.7
Hyperventilation on air	16.6	7.1	−1.5	37.9
Hyperventilation on air in combination with albumin infusion	16.3	10.3	5.3	39.5

* Mean values for three subjects.

mean D_L to 39.5 ml. per minute per mm. Hg, essentially the same increase as with hyperventilation alone.

In summary, albumin infusions, intended to produce pulmonary vascular engorgement, did not enhance the effect of hyperventilation on D_L, as measured by the steady state method.

B. Studies with D_L determined by the breath holding technique

As shown in Table VIII, 19 supine subjects had a mean D_L of 35.7 ± 8.4 ml. per minute per mm. Hg at rest. Eight of these subjects, with a mean D_L of 38.6 ± 9.7 ml. per minute per mm. Hg supine, had a mean D_L of 34.8 ± 5.9 ml. per minute per mm. Hg when seated (Table IX). This value agrees closely with the mean D_L of 31.7 ± 4.4 ml. per minute per mm. Hg which Ogilvie, Forster, Blakemore and Morton found in eight young male seated subjects (5). Three subjects (ages 10, 10 and 8) were excluded from their group of 11 subjects in determining this mean, in order to make the group comparable to ours in age and size. In the present group of eight subjects, the difference between D_L in the supine and seated positions is not statistically significant.

The procedures required to prepare the subject for the collection of arterial blood samples and determination of cardiac output produce a small but unavoidable amount of discomfort. To study the effect of this discomfort and accompanying apprehension on the control level of D_L, the D_L was determined in 11 subjects: *a*) before any procedures, *b*) immediately after the arterial needle and venous catheter were put in place, and *c*) after the usual 30 minute waiting period. Mean values were 39.0 ± 8.0, 40.0 ± 10.1 and 38.2 ± 9.9 ml. per minute per mm. Hg, respectively, and are not significantly different.

TABLE VIII

Breath holding D_L determinations in 19 normal supine subjects at rest

Subject	Age	Surface area	Number of determinations	Mean alveolar volume	Mean D_L
		M.²		*ml.*	*ml./min./mm. Hg*
P. B.	23	2.10	2	5,570	32.9
H. S.	22	1.72	4	4,450	35.0
B. L.	23	2.14	1	5,570	47.8
C. H.	21	2.14	4	5,090	39.4
Jo. W.	21	2.08	8	5,200	35.7
C. W.	24	1.87	4	3,850	30.0
J. B.	21	2.12	5	4,430	28.0
J. P.	22	2.04	4	5,110	52.4
D. G.	22	2.06	4	5,240	52.2
J. C.	23	1.86	4	3,780	29.8
F. D.	24	2.10	6	6,040	40.9
R. C.	23	2.02	4	6,050	44.2
J. W.	31	1.88	4	5,400	34.6
L. W.	23	2.28	4	4,900	30.1
G. P.	25	2.02	3	5,870	32.6
J. O.	23	1.79	3	4,440	31.1
H. T.	22	2.14	3	5,460	27.2
S. S.	22	1.70	3	3,490	22.8
F. A.	21	1.76	3	3,980	32.4
Mean	23	2.00		4,940±785	35.7±8.4

TABLE IX

Breath holding D_L determinations in eight normal seated subjects at rest

Subject	Age	Surface area	Alveolar volume	Seated D_L	Mean supine D_L*
		M.²	*ml.*	*ml./min./mm. Hg*	
C. H.	21	2.14	4,700	32.2	39.4
Jo. W.	21	2.08	5,730	34.2	35.7
C. W.	24	1.87	4,140	27.9	30.0
J. B.	21	2.12	5,000	32.2	28.0
T. P.	22	2.04	5,380	40.4	52.4
O. G.	22	2.06	6,100	42.5	52.2
J. C.	23	1.86	4,380	27.8	29.8
F. D.	24	2.10	6,400	41.4	40.9
Mean	22	2.03	5,230±815	34.8±5.9	38.6±9.7

* From Table VIII.

Effect of exercise and increased cardiac output on the breath holding D_L

D_L and cardiac output were determined in eight subjects while at rest, then during exercise, and finally at rest with cardiac output increased by drugs (Table X). During exercise, mean cardiac output increased to 13.2 ± 2.1 L. per minute from 6.6 ± 1.0 L. per minute at rest while mean D_L increased to 48.9 ± 10.5 ml. per minute per mm. Hg from 39.8 ± 9.7 ml. per minute per mm. Hg at rest. When the cardiac output was increased to 11.9 ± 3.6 L. per minute by drugs, however, the mean D_L did not increase from the resting value.

Four of these subjects also had determinations of cardiac output and D_L during exercise at the same time that their cardiac output was already increased by drugs to determine whether these drugs might have some action which would tend to prevent D_L from increasing (Table XI). The result was not entirely clear-cut. In each sub-

ject, exercise still caused an increase in D_L, although the increase was less than that produced by exercise before the administration of drugs.

The effect of increasing cardiac output by release of arterial tourniquets which had occluded blood flow to the lower extremities for the preceding 15 minutes was also investigated in three subjects. As indicated in Table XII, the mean cardiac output was almost doubled, but there was little change in D_L.

In summary, breath holding D_L was considerably increased, as expected, by exercise which doubled the cardiac output, but doubling the output by drugs or tourniquet release had little effect on D_L.

TABLE X

Breath holding D_L, alveolar volume and cardiac output in eight supine subjects at rest, during exercise, and with the cardiac output increased by drugs

Subject	Condition	Alveolar volume	C.O.	D_L
		ml.	*L./min.*	*ml./min./mm. Hg*
B. L.	Rest	5,570	7.0	47.8
	Exercise	5,990	12.4	57.5
	Drugs*	5,890	14.9	49.6
C. H.	Rest	5.040	6.3	38.0
	Exercise	4,880	13.3	43.4
	Drugs	4,950	9.9	40.7
Jo. W.	Rest	4,750	8.7	33.1
	Exercise	5,270	15.9	47.5
	Drugs	5,410	8.1	33.1
C. W.	Rest	3,760	6.0	28.4
	Exercise	4,100	12.4	36.9
	Drugs	3,500	9.4	27.4
T. P.	Rest	5,100	5.7	52.5
	Exercise	5,460	11.5	64.3
	Drugs	5,160	15.4	45.2
F. D.	Rest	6,080	5.6	39.7
	Exercise	6,330	16.7	48.3
	Drugs	6,150	10.6	42.3
D. G.	Rest	4,780	6.4	50.8
	Exercise	5,330	10.5	58.4
	Drugs	5,030	17.6	53.7
J. C.	Rest	3,930	7.1	28.0
	Exercise	4,450	13.1	34.8
	Drugs	4,060	9.0	29.0
Mean	Rest	4,880±770	6.6±1.0	39.8± 9.7
	Exercise	5,230±740	13.2±2.1	48.9±10.5
	Drugs	5,020±880	11.9±3.6	40.1± 9.5

* Atropine and norepinephrine administration as described in Methods.

TABLE XI

Breath holding D_L and cardiac output in four supine subjects at rest, during exercise, with cardiac output increased by drugs, and with exercise while the cardiac output was increased by drugs

Condition	C.O.*	D_L*
	L./min.	*ml./min./mm. Hg*
Rest	6.2 ± 0.7	42.8 ± 11.3
Exercise	13.0 ± 2.7	51.5 ± 12.9
Drugs†	13.2 ± 4.0	42.5 ± 10.6
Exercise with drugs†	16.6 ± 2.6	46.5 ± 9.0

* Means of four subjects.
† Atropine and norepinephrine administration as described in Methods.

Effect of increased ventilation rate on the breath holding D_L

Even though the breath is being held during the determination of D_L, exercise is accompanied by an increase in ventilation rate in the period preceding the breath holding period. The possibility that increased ventilation before the breath holding period could change D_L was studied by having five subjects hyperventilate for a period of five or six minutes immediately prior to the determination of D_L (Table XIII). The mean D_L at rest was 36.4 ± 8.0 ml. per minute per mm. Hg and after hyperventilation was 35.5 ± 7.4 ml. per minute per mm. Hg. Ogilvie, Forster, Blakemore and Morton reported comparable results in a similar study (5).

TABLE XII

Effect of blood pH changes on the breath holding D_L in three supine subjects

Condition	C.O.*	Arterial* pH	D_L*
	L./min.		*ml./min./mm. Hg*
Rest		7.42	36.3
Arterial tourniquets on the lower extremities	4.4	7.42	33.8
30–40 seconds after the tourniquets were released	8.5	7.35	35.1
After NH_4Cl administration		7.38	35.8
Arterial tourniquets on the lower extremities after NH_4Cl administration		7.38	35.0
30–40 seconds after the tourniquets were released		7.34	37.8

* Means of three subjects.

TABLE XIII

Effect of increased ventilation rate on the breath holding D_L in five supine subjects

Condition	Alveolar* volume	C.O.*	D_L*
	ml.	*L./min.*	*ml./min./mm. Hg*
Rest	4,460 ± 880	7.0 ± 1.2	36.4 ± 8.0
Hyperventilation†	4,290 ± 820	6.0 ± 4.0	35.5 ± 7.4

* Means of five subjects.
† Hyperventilation for five to six minutes immediately before the breath holding period.

Effect of a combination of increased cardiac output and increased ventilation rate on the breath holding D_L

The breath holding D_L was not changed by either increased ventilation rate prior to the test or increased cardiac output alone. To mimic exercise more closely, two subjects were studied, first at rest, then with cardiac output increased by drugs, and finally with hyperventilation while the cardiac output was increased by drugs. The mean D_L did not change significantly when the mean cardiac output was increased from 6.0 to 10.1 L. per minute by drugs. With the mean cardiac output increased to 10.1 L. per minute, the subjects hyperventilated for five or six minutes immediately prior to the breath holding period, but this also failed to change D_L significantly.

Effect of blood pH changes on the breath holding D_L

Exercise causes a reduction in the blood pH, more in mixed venous than arterial blood. An attempt was made to simulate this change without actual exercise by the use of arterial tourniquets on the thighs and administration of NH_4Cl. Release of arterial tourniquets which have made the legs ischemic for 15 minutes not only increases the cardiac output (18), but also reduces blood pH because a large volume of hypoxic, relatively acidic, blood enters the venous side of the circulation from the previously occluded region (26). To reinforce this drop in pH, subjects were also studied after they were prepared by the oral administration of 20 Gm. of NH_4Cl during the course of the day before the last part of the experiment was carried out. D_L and arterial blood pH, therefore, were determined in three subjects: *a*) before arterial tourniquets were applied to the lower extremities, *b*) while arterial tourniquets occluded blood flow to the lower extremities, *c*) within 30 to 60 seconds after release of the arterial tourniquets, and *d*) under all three of the aforementioned conditions after the administration of NH_4Cl. In this study, as in the other studies where pH was determined in conjunction with the breath holding D_L, the arterial blood sample for pH determination was withdrawn from the indwelling arterial needle over the entire 10 second breath holding period. There appears to be no relation between D_L and changes in arterial blood pH brought about by these procedures (Table XII). In addition, a trial was made in two subjects of the effect of maintaining the arterial blood pH above 7.50 by voluntary overbreathing during exercise. This procedure had no apparent effect on the increase in D_L during exercise, as measured by the single breath method.

In summary, exercise causes a reduction in the blood pH, but reduction of the blood pH by experimental methods did not increase the D_L as does exercise. On the other hand, when the reduction in blood pH, usually caused by exercise, was prevented by overventilation, the increase in D_L with exercise was not altered.

Effect of reduction in mixed venous blood oxygen content on the breath holding D_L

Exercise reduces the oxygen content of mixed venous blood without much change in arterial blood oxygen. Conceivably, venous hypoxia could affect the diffusing capacity by changing the resistance of precapillary vessels in the lung and so changing the distribution of blood to the capillaries. To produce venous, but not arterial, hypoxia without exercise, the following procedure was employed. Two subjects breathed 10 per cent oxygen for 10 minutes, lowering the arterial blood oxygen saturation to between 60 and 70 per cent, as estimated by an ear oximeter,[5] and presumably reducing the mixed venous blood oxygen. At the end of this time, for measurement of D_L by the breath holding method, the subject made the usual full expiration followed by a maximal inspiration of the carbon monoxide mixture, which contained 21 per cent oxygen. For the 10 seconds of the measurement, before

[5] Waters Conley.

significant recirculation occurred, this procedure achieved some degree of mixed venous hypoxia with substantially normal arterial blood oxygen saturation. This procedure, tried in two subjects, had a negligible effect on D_L, and increasing the cardiac output with drugs at the same time caused no further change. These results are comparable to the results of a similar experiment reported by Cander and Forster (27).

DISCUSSION

It is well established that exercise increases the pulmonary diffusing capacity. In this study, as in previously reported studies (1–5), exercise caused an increase in D_L, both breath holding and steady state, in all subjects where D_L was determined before and during exercise; and, as others have also observed (5), there was a proportionally greater increase in the steady state D_L than in breath holding D_L. The mechanism of this increase in D_L with exercise was studied by measuring the apparent pulmonary diffusing capacity in situations where one or more of the effects of exercise were reproduced experimentally.

Krogh (1) suggested that change in a stagnant plasma layer in the capillaries could be the mechanism by which exercise increases D_L. Change in the capillary wall permeability or capillary size brought about by lung volume change was suggested by Barcroft (28) as an explanation for the increase in D_L with exercise. Forster (29) has pointed out that it is reasonable to assume that the surface area of the capillary bed increases with exercise, either by dilatation of patent vessels or opening of previously closed ones, as a result of vasomotion or hemodynamic forces. Roughton (12) found that the volume of blood in the pulmonary capillary bed is increased with exercise.

The cardiac output increases during exercise and it has been considered that the increase in D_L is dependent in some way upon this change. This factor was studied by increasing the cardiac output in subjects otherwise at rest and measuring D_L. The cardiac output was increased in groups of subjects by three somewhat different methods: *a*) drugs, *b*) albumin infusion, and *c*) release of arterial tourniquets previously occluding blood flow into the lower extremities. In this study, neither the breath holding nor steady state D_L were increased when the cardiac output was increased by methods other than exercise. In one group (Table III), the mean cardiac output was the same during exercise as when drugs were given, but even though D_L was markedly increased by exercise, D_L did not increase over the resting level when the cardiac output was increased by drugs. In all, 67 simultaneous determinations of cardiac output and steady state D_L were made in 24 subjects under different experimental conditions. There is no significant correlation between the two variables ($r = 0.219$). This is supported by the observation of Johnson, Stein and Kimbel (30) who found that the breath holding D_L of several patients with increased cardiac output due to hyperthyroidism was not significantly different from the D_L found when they had normal cardiac output after they were made euthyroid by therapy.

The effect of increasing pulmonary vascular (pulmonary arterial and capillary) pressure was also investigated. Increased pulmonary vascular pressure is probably not the cause of an increased D_L with exercise since D_L increases significantly with moderate exercise, whereas in normal man there is little or no increase in pulmonary arterial pressure with moderate exercise (31, 32). This study was made, however, to determine whether an increase in D_L could be produced by procedures designed to increase pulmonary vascular pressures. With experiments in which the ventilation rate was substantially unchanged, increase in pulmonary vascular pressures, as reflected by a fairly large increase in the mean central venous pressure, was accompanied by only a small increase in the steady state D_L. Lewis, Forster and Beckman (23) did not find a significant increase in the breath holding D_L with inflation of a G suit.

Increases in ventilation rate, however, whether or not produced by exercise, were uniformly found to be associated with increases in steady state D_L. Hyperventilation alone, without any change in the cardiac output, was accompanied by an increase in steady state D_L in all subjects studied, whether they hyperventilated while breathing air or 5 per cent CO_2. The increase in steady state D_L with hyperventilation in one group of subjects (Table V) was about the same

as the increase produced by exercise in another group of subjects (Table III) where the increase in mean ventilation rate was nearly the same. There is a highly significant correlation ($r = 0.787$) between steady state D_L and ventilation rate in the 67 determinations on 24 subjects mentioned above in whom the correlation between D_L and cardiac output was not significant. Taken alone, these data suggest that the effect of ventilation rate on steady state D_L is sufficient to account for the increase in D_L which occurs with exercise. Increases in breath holding D_L, however, also occur with exercise when the rate of ventilation cannot be a factor during the test period itself because the breath is being held. Hyperventilation before the breath holding period, to mimic the course of ventilation during exercise, was without effect on D_L. Similar results have been reported previously (5). It would appear that the increase in breath holding D_L which occurs with exercise depends upon some mechanism other than increase in cardiac output or ventilation rate.

An attempt was made to enhance the effect of hyperventilation on D_L by simultaneously increasing cardiac output or by producing pulmonary vascular engorgement. The increase in steady state D_L produced by hyperventilation, however, was not enhanced by simultaneous pulmonary vascular engorgement. In addition, the breath holding D_L, which was not increased by hyperventilation alone before the test period, likewise failed to increase when hyperventilation was combined with increase in cardiac output caused by drugs.

Exercise reduces pH and pO_2 and increases pCO_2 of mixed venous blood. Conceivably, such changes could affect the precapillary vascular system of the lungs in such a way as to alter the distribution of blood and increase D_L. For this reason, the effect on the breath holding D_L of changes in pH and gas content of mixed venous blood was studied in small groups of subjects. Reducing pH did not cause an increase in breath holding D_L and, likewise, partially counteracting the acidosis of exercise by hyperventilation did not prevent an increase in D_L from occurring with exercise. Forster and co-workers (13, 29) found only a slight increase in breath holding D_L when 6.0 per cent CO_2 was included in the inspired mixture, but a more significant increase in breath holding D_L of 20 per cent in nine subjects who had breathed 7.5 per cent CO_2 for 10 minutes. Observations were also made on the effect of reducing mixed venous pO_2. The breath holding D_L was neither increased by the procedure which was designed to reduce the mixed venous pO_2 while allowing a normal arterial pO_2, nor by combination of this procedure with an increased cardiac output. The procedure which probably produced changes in the mixed venous blood most like those of exercise was the release of tourniquets from ischemic lower extremities, which increases cardiac output and releases blood with a reduced pH and pO_2 and an increased pCO_2 into the venous side of the circulation. This, however, failed to increase D_L as measured by the breath holding method. Breath holding D_L was determined about 30 to 40 seconds after release of tourniquets, and it is possible that the changes caused by this procedure were not present for sufficient time to increase D_L.

In summary, the breath holding D_L was not increased significantly by any procedure except exercise, while the steady state D_L was increased by hyperventilation alone to about the extent that it was increased by exercise with a similar ventilation rate.

There are other factors which are known to be important in determining the actual value of D_L, but are not applicable in this study. The values of D_L reported here are for apparent pulmonary diffusing capacity of the lung as a whole, which includes the factors of resistance to passive diffusion of carbon monoxide through the "membrane" and resistance to reaction with hemoglobin. Roughton, Forster and co-workers (12, 33–36) have measured the reaction rate of CO with hemoglobin and devised a method for determining separately the diffusing capacity of the membrane (D_m). While the reaction rate of CO with hemoglobin must be determined in order to measure D_m, the apparent D_L can be measured without consideration for kinetics of the CO+Hb reaction, though it is obviously playing a part in the actual value obtained. The reaction rate of CO+Hb would have been applicable in this study only if it were being considered as a possible mechanism by which exercise increases D_L or if it were being changed by procedures used

to mimic the effects of exercise. There is no indication that a significant change in reaction rate was produced in this way. Reaction rate can be affected by changes in mean pO_2 of pulmonary capillary blood (37), but the present experimental procedures probably altered this little. In a normal subject breathing 21 per cent oxygen, the oxygen tension of pulmonary capillary blood probably increases so rapidly when the blood is exposed to alveolar gas tensions (38), that the mean capillary pO_2 is not greatly altered by moderate changes in the pO_2 of pulmonary arterial blood. Even if the mean capillary pO_2 were calculated as the arithmetic mean of pulmonary arterial and systemic arterial blood pO_2 the present experimental procedures would have produced little change. From the data in Table III, it can be calculated that the mean A–V oxygen difference was 3.7 volumes per cent for subjects at rest, 6.0 during exercise, and 2.2 when the cardiac output was increased by drugs. For normal subjects these values would correspond to pulmonary arterial pO_2 values of approximately 48, 40 and 60 mm. Hg, respectively, (pH 7.30). The greatest difference in mean capillary pO_2 between these groups should have been less than 10 mm. Hg, and the effect of this on the reaction rate of carbon monoxide with hemoglobin is negligible for present purposes (37).

Steady state D_L determinations are influenced by the mixed venous COHb concentration (11, 12) and to determine D_L as accurately as possible, this must be taken into account. The present study has been concerned primarily with factors which change D_L rather than with the absolute value of D_L. For the reasons already given, therefore, "back pressure" does not seem to be significant in the interpretation of the results of this study and no correction was made for capillary pCO. In the case of the breath holding D_L, the effect of capillary pCO has also been found to be almost insignificant at near normal alveolar O_2 tensions (11, 39).

The answer to the question of why D_L increases with exercise is incomplete. By these methods, changes in cardiac output and pulmonary vascular pressures are without significant effect. With the steady state method, ventilation rate is of great importance and in the use of this method for investigative purposes, consideration must be given to the effect of changes in ventilation rate. The mechanism by which increased ventilation rate causes an increase in steady state D_L has not been determined. During exercise or with hyperventilation, the mean volume of the lung is somewhat increased and it has been suggested in the past that increase in lung volume is the mechanism by which exercise causes an increase in D_L (28). Grape and Tyler (40) found that the steady state D_L increased when the alveolar volume was increased, but the amount of rise they observed is not sufficient to account for the rise which occurs with exercise. Increased ventilation rate may possibly increase the steady state D_L by causing an increase in the perfused area of the pulmonary capillary bed or by causing a more even ventilation-perfusion ratio throughout the lung (29). The data available from this study do not bear on these possibilities.

SUMMARY

1. A study has been made of the means by which exercise increases the pulmonary diffusing capacity for carbon monoxide (D_L) in normal subjects, using both the steady state and breath holding techniques.

2. Increases in cardiac output produced by means other than exercise caused no significant change in D_L as measured by either the steady state or breath holding technique.

3. Steady state D_L was found to be very sensitive to the ventilation rate, and by hyperventilation alone, D_L was increased as much as by exercise at the same minute ventilation. Breath holding D_L was not affected by hyperventilation preceding the measurement.

4. Although the effect of increased ventilation rate could explain the increase in steady state D_L with exercise, an adequate explanation for the increase in breath holding D_L which occurs during exercise was not obtained.

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IN VIVO STUDIES OF 5-HYDROXYINDOLE METABOLISM IN PATIENTS WITH HEPATIC CIRRHOSIS AND IN RATS * †

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Widespread interest in 5-hydroxytryptamine (5-HTA) as a biologically active amine and the close association between this substance and malignant carcinoidosis have led to detailed studies of the 5-hydroxyindole pathway of tryptophan metabolism in man and experimental animals (1-3). Tryptophan is apparently hydroxylated to form the serotonin precursor, 5-hydroxytryptophan (5-HTP) (1, 4). In the presence of a specific, pyridoxine-dependent decarboxylase, 5-HTP is readily decarboxylated in several tissues to form 5-HTA (5). Monoamine oxidase, which is found principally in gut, lung, liver, kidney and brain, catalyzes the oxidative deamination of 5-HTA to form 5-hydroxyindoleacetic acid (5-HIAA) (6, 7).

Since both 5-HTP decarboxylase and monoamine oxidase have been demonstrated in liver tissue *in vitro,* investigation of 5-hydroxyindole metabolism in patients with hepatic cirrhosis was undertaken. The present report summarizes studies of 5-HTA and 5-HIAA excretion in the urine following the intravenous administration of various 5-hydroxyindole compounds in human subjects with and without hepatic cirrhosis and in rats.

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MATERIALS AND METHODS

1. Endogenous excretion of 5-HIAA in human subjects. The urinary excretion of endogenous 5-HIAA was measured in one or more total 24 hour urine collections obtained from 10 healthy adults, 75 hospitalized patients without hepatic disease, renal disease or known sprue syndrome (8), 35 patients hospitalized with hepatic cirrhosis and two patients with malignant carcinoidosis. None of the subjects had clinical evidence of renal disease. All had 24 hour urine volumes greater than 500 ml. and blood urea nitrogens of less than 20 mg. per cent. None of the subjects were receiving phenothiazine derivatives (9) or a banana diet (10) during the period of study.

2. Intravenous administration of 5-hydroxyindole compounds to human subjects. Studies of the urinary excretion of 5-HTA and 5-HIAA following the intravenous administration of various 5-hydroxyindole compounds were carried out in 21 control subjects, in 20 patients with decompensated hepatic cirrhosis and in seven patients with surgically documented extrahepatic obstruction with no evidence of significant intrahepatic disease. 5-HTP [1] (0.33 mg. of L form per Kg.) or 5-HIAA (0.2 mg. per Kg.) was diluted in 250 to 300 ml. of isotonic saline and administered over a period of one hour in the early morning to subjects in the fasting state. 5-HTA [2] (0.2 mg. of 5-HTA base per Kg.) was diluted in 500 to 1,000 ml. of isotonic saline and administered intravenously over a period of two to four hours. At least 48 hours was allowed to elapse between infusions whenever the same subject received more than one intravenous infusion. The total urinary excretion was collected in six hour aliquots for at least 24 hours before and after each intravenous infusion. Results were calculated as percentage of the administered 5-hydroxyindole compound which was recovered in the urine as 5-HIAA or 5-HTA.

3. Studies in rats. The urinary excretion of 5-HIAA and 5-HTA following the administration of various 5-hydroxyindole compounds was studied in male Sprague-Dawley rats weighing 300 to 500 Gm. The animals were maintained on regular laboratory Purina chow and water *ad libitum.* Each rat served as his own control. Sodium orthoaminobenzoate (NaOAB), 1 Gm. per Kg.

[1] Administered as 5-hydroxy-DL-tryptophan, supplied by Abbott Laboratories.

[2] Administered as serotonin creatinine sulfate, supplied by Abbott Laboratories.

TABLE I

The endogenous urinary excretion of 5-hydroxyindoleacetic acid in human subjects

Group	Number of subjects	Number of determinations	Range	Average
			mg./24 hrs.	*mg./24 hrs.*
Healthy adults	10	27	2.2– 6.7	4.0 ± 1.4
Hospitalized patients without liver or renal disease	75	132	1.5–12.4	4.3 ± 2.4
Hepatic cirrhosis without encephalopathy	19	39	1.9–13.4	6.7 ± 3.3
Hepatic cirrhosis with encephalopathy	16	34	2.1–13.7	6.1 ± 3.1
Carcinoid syndrome	2	12	45–360	154– ± 78

body weight, was injected intraperitoneally together with a given 5-hydroxyindole compound. Since NaOAB is rapidly conjugated to form a glucuronide, excessive amounts were administered in this study in an attempt to inhibit competitively the conjugation of serotonin. At least 48 hours before and again 48 hours after NaOAB was injected together with the 5-hydroxyindole compound, each rat received the same dose of the 5-hydroxyindole compound together with an equivalent amount of sodium chloride. The 5-hydroxyindoles administered in this fashion included 5-HTP (25 mg. of the L form per Kg.), 5-HTA (25 mg. of the base per Kg.) and 5-HIAA (20 mg. per Kg.). The animals were kept in metabolic cages and the total urinary excretion was collected separate from feces for 24 hours after each injection.

4. Methods. Aliquots of all urine specimens were kept frozen until analyzed. The urinary excretion of 5-HIAA was measured by the colorimetric method of Udenfriend, Titus and Weissbach (11). Urine samples containing more than 25 μg. per ml. of 5-HIAA were appropriately diluted. The 5-HTA content of urine was estimated fluorimetrically by a modification (12) of the method of Udenfriend, Weissbach and Clark (13). All determinations were carried out in duplicate and were repeated whenever the results varied more than 10 per cent in the duplicate samples. The accuracy of all urine collections was estimated by measurement of urinary excretion of creatinine (14). Paper chromatograms of urinary indoles were prepared by the method of Jepson (15).

RESULTS

1. Studies in human subjects

Results of the urinary excretion of endogenous 5-HIAA are summarized in Table I. The average excretion of 5-HIAA in patients with hepatic cirrhosis with or without evidence of encephalopathy was not significantly greater than that observed in healthy adults or patients without liver disease. The two subjects with metastatic carcinoid tumors excreted strikingly increased amounts of 5-HIAA in the urine. 5-HTA was present in small amounts in the two carcinoid patients but was never observed in other subjects unless they had received intravenous 5-HTP.

When 5-HTP was administered to control subjects an average of 32 per cent (13 to 42 per cent) of the administered 5-HTP was recovered in the urine as 5-HIAA (Table II). The recovery of administered 5-HTP as urinary 5-HIAA averaged 62 per cent (25 to 105 per cent) among cirrhotic subjects (Table II). The difference between the two groups is significant ($p = < 0.001$). Flapping tremors were noted in all six of the cir-

TABLE II

Summary of studies of 5-hydroxyindole metabolism in human subjects

Group	Number of subjects	Compound administered	Compound measured in urine	Percentage of administered compound in urine
				± *S.D.*
Controls	21	5-HTP	5-HIAA	32 ± 5.3
Cirrhosis	20	5-HTP	5-HIAA	62 ± 19.2
Obstruction	7	5-HTP	5-HIAA	31 ± 14.0
Controls	9	5-HTP	5-HTA	11 ± 4.0
Cirrhosis	8	5-HTP	5-HTA	15 ± 7.8
Controls	3	5-HTA	5-HIAA	59 ± 10.1
Cirrhosis	3	5-HTA	5-HIAA	60 ± 7.1
Controls	7	5-HIAA	5-HIAA	53 ± 7.1
Cirrhosis	6	5-HIAA	5-HIAA	64 ± 15.5

TABLE III

The effect of sodium orthoaminobenzoate (NaOAB) on the recovery of administered 5-hydroxyindole compounds in rats

Number of rats	5-Hydroxyindole administered	Additional substance administered	% of administered 5-hydroxyindole recovered as urinary 5-HIAA	Urinary creatinine
			± *S.D.*	± *S.D.*
9	5-HTP	NaCl	55.2 ± 8.8	22.6 ± 3.2
9	5-HTP	NaOAB	83.5 ± 7.9	23.7 ± 4.6
9	5-HTP	NaCl	51.9 ± 9.1	23.1 ± 3.8
5	5-HTA	NaCl	38.2 ± 7.4	20.8 ± 4.3
5	5-HTA	NaOAB	68.0 ± 10.5	22.6 ± 3.9
5	5-HTA	NaCl	39.2 ± 9.5	20.9 ± 3.6
9	5-HIAA	NaCl	76.0 ± 11.8	25.0 ± 4.1
9	5-HIAA	NaOAB	71.0 ± 12.2	25.0 ± 2.9

rhotic subjects in whom the recovery of 5-HTP as urinary 5-HIAA exceeded 70 per cent. Four of these subjects were confused or stuporous, and frank hepatic coma was present in one. The recovery of administered 5-HTP as urinary 5-HIAA in patients with extrahepatic biliary tract obstruction was not different from that observed in control subjects (Table II). The differences in recoveries in the three groups are summarized in Figure 1. 5-HTP was administered on more than one occasion to four control and two cirrhotic subjects. Reproducible recoveries of urinary 5-HIAA were obtained in each of these subjects. The recoveries of administered 5-HTP as urinary 5-HTA in control subjects did not differ from those observed in patients with hepatic cirrhosis (Table II). The average urine volume and creatinine excretion following 5-HTP administration was not different in the two groups.

The intravenous administration of 5-HTP resulted in mild nausea lasting for 10 to 60 minutes in nearly all of the subjects studied. A transient episode of vomiting occurred in only two subjects. None experienced diarrhea. In the cirrhotic patients with impending or frank hepatic coma, administration of 5-HTP had no obvious clinical effect on the flapping tremors or mental state.

When 5-HTA was administered to control and cirrhotic subjects significant amounts of 5-HTA were not found in the urine. The recoveries of administered 5-HTA and 5-HIAA as urinary 5-HIAA were not different in the control and cirrhotic subjects (Table II). Intravenous infusion of 5-HTA resulted in venospasm with local pain and slowing of the infusion, but produced no systemic effects.

No consistent differences were observed in paper chromatograms of indolic compounds which were prepared from the urines of control and cirrhotic subjects who had received 5-hydroxyindole compounds intravenously.

2. Studies in rats

The results of intraperitoneal administration of 5-hydroxyindole compounds to rats together with NaOAB or sodium chloride as a control are summarized in Table III and in Figure 2. In nine rats, the administration of NaOAB together with 5-HTP resulted in a significantly ($p = < 0.001$) greater excretion of urinary 5-HIAA than was observed when 5-HTP was administered together

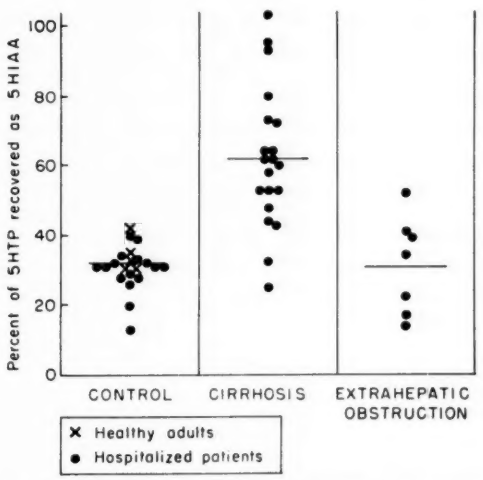

FIG. 1. RECOVERY OF ADMINISTERED 5-HTP AS URINARY 5-HIAA IN HUMAN SUBJECTS

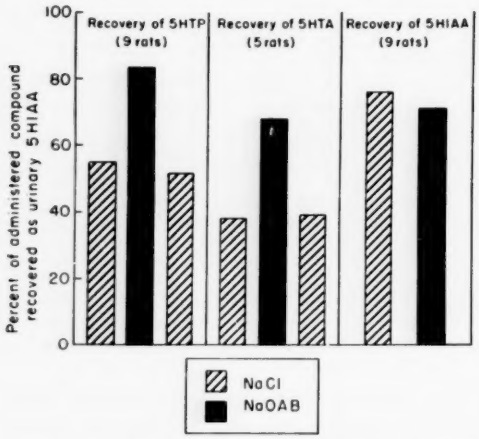

FIG. 2. THE EFFECT OF SODIUM ORTHOAMINOBENZOATE (NaOAB) ON THE METABOLISM OF 5-HYDROXYINDOLE COMPOUNDS IN RATS

with NaCl. The urinary excretion of 5-HTA was consistently diminished when NaOAB was administered together with 5-HTP. Addition of NaOAB directly to urine did not interfere with the measurement of 5-HTA or 5-HIAA.

Only five of 21 rats survived the simultaneous intraperitoneal injection of 5-HTA and NaOAB or NaCl. In general the combination of 5-HTA and NaOAB proved fatal more often than 5-HTA and NaCl. Focal areas of necrosis in the kidneys and pulmonary edema were regularly observed at necropsy. In the five surviving rats the administration of 5-HTA together with NaOAB resulted in a significantly ($p < 0.001$) greater urinary excretion of 5-HIAA than was observed following the injection of 5-HTA with NaCl. Significant amounts of 5-HTA were not found in the urine.

In nine rats the urinary excretion of 5-HIAA was not altered when 5-HIAA was administered intraperitoneally with NaOAB or NaCl. In all of the rats studied the urinary excretion of creatinine remained constant throughout all the experiments.

DISCUSSION

No striking alteration was observed in the urinary excretion of endogenous 5-HIAA in the patients with hepatic cirrhosis whether or not impending or frank hepatic coma was present. It has been suggested that 5-HIAA excretion might be diminished in cirrhotic patients as a result of impaired formation of 5-HTP in the liver, and, on this basis, a deficiency of serotonin has been postulated in the pathogenesis of hepatic coma (16). The site of tryptophan hydroxylation, however, is as yet unknown, and there is no evidence that significant formation of 5-HTP from tryptophan occurs in the liver (4). The data presented here do not support the concept that 5-HIAA excretion may be decreased in patients with hepatic cirrhosis. Why the urinary excretion of endogenous 5-HIAA is not significantly different in cirrhotic and control subjects while the metabolism of exogenous 5-HTP appears to be altered in hepatic cirrhosis remains unexplained. It is possible that the urinary excretion of 5-HIAA after the administration of 5-HTP may not represent a true reflection of endogenous 5-hydroxyindole metabolism in patients with hepatic cirrhosis.

The recoveries of 5-HIAA in the urine of control subjects following the administration of 5-HTP, 5-HTA and 5-HIAA are similar to those previously reported in normal humans (3). It would appear, however, that the metabolism of exogenous 5-HTP may be altered in patients with hepatic cirrhosis. When 5-HTP was administered to a group of cirrhotic patients the resulting excretion of 5-HIAA in the urine was greater than that observed in control subjects and in patients with extrahepatic biliary tract obstruction. The cirrhotic patients selected for this study were all hospitalized primarily because of liver disease. All were jaundiced or had ascites. Although there was no distinct correlation between the severity of liver disease and the recovery of administered 5-HTP as urinary 5-HIAA, the six cirrhotic patients with the greatest urinary excretion of 5-HIAA following 5-HTP administration all showed clinical evidence of impending or frank hepatic coma.

There are several possible explanations for the greater than normal recovery of urinary 5-HIAA observed in cirrhotic patients after intravenous infusion of 5-HTP. It would appear that 5-HIAA metabolism is probably not altered in hepatic cirrhosis since the recoveries of urinary 5-HIAA after the administration of exogenous 5-HIAA were similar in control and cirrhotic subjects. Whether or not the metabolism of administered 5-HTA is

altered in patients with cirrhosis of the liver is not clear from this study. Because of venous spasm and pain at the site of infusion, 5-HTA was infused at slow and variable rates to only a few subjects. Since the urinary excretion of 5-HIAA after 5-HTP administration was similar in control subjects and patients with extrahepatic biliary tract obstruction, it seems unlikely that diminished excretion of 5-hydroxyindole compounds in the bile would play a role in the altered metabolism of exogenous 5-HTP observed in patients with hepatic cirrhosis. Although the D-form of 5-HTP may be oxidized by D-amine oxidase *in vitro* (6), the amount of 5-HIAA which may thus be produced *in vivo* from D-5-HTP is as yet unknown. The possibility exists, nevertheless, that the metabolism of the D form of 5-HTP might be altered in hepatic decompensation in such a way as to explain the results obtained in cirrhotic subjects. The data presented in this study, however, do not provide evidence for evaluation of this possibility.

Incubation of 5-hydroxyindole compounds with liver *in vitro* and chromatographic examination of urinary indoles excreted by carcinoid patients and by animals infused with 5-hydroxyindole compounds suggest that 5-HTA may be metabolized by mechanisms other than oxidative deamination (2, 4, 17–20). *In vitro* studies have demonstrated conjugation of 5-HTA in the liver to form serotonin-*o*-sulfate (17). The formation of serotonin-*o*-glucuronide has been demonstrated *in vivo* in animals after the administration of 5-HTA (19, 20).

From the above studies it appears reasonable to postulate that the greater than normal excretion of urinary 5-HIAA following the infusion of 5-HTP in hepatic cirrhosis may be a result of impaired conjugation of 5-HTA. If hepatic conjugation of 5-HTA were impaired, more 5-HTA might be available for metabolism of 5-HIAA. The effect of sodium orthoaminobenzoate (NaOAB) on the excretion of 5-HIAA following the administration of 5-HTP or 5-HTA to rats is of interest in this regard. NaOAB is rapidly conjugated to form a glucuronide (21), and when present in great excess may impair the formation of other glucuronides. Administration of 1 Gm. of NaOAB per Kg. body weight to rats results in a maximal urinary excretion of glucuronic acid (22). *In vitro* studies suggest that the formation of glucuronide conjugates may be competitively inhibited by the presence of NaOAB or similar compounds (23).

The data indicate that in the presence of excess NaOAB the urinary excretion of 5-HIAA following the administration of 5-HTP or 5-HTA is significantly greater than that observed when these 5-hydroxyindole compounds are administered alone. NaOAB appeared to have no effect on the metabolism of exogenous 5-HIAA in these animals. The data presented do not demonstrate that NaOAB administration results in diminished hepatic conjugation of 5-HTA, nor has impairment of 5-HTA conjugation been shown in patients with hepatic cirrhosis. These studies, however, indirectly support the concept that 5-HTA conjugation may occur in the liver and are consistent with the hypothesis that with impairment of 5-HTA conjugation, larger amounts of 5-HTA may be available for metabolism to 5-HIAA.

SUMMARY

1. The urinary excretion of 5-hydroxyindoleacetic acid and 5-hydroxytryptamine was studied in patients with decompensated hepatic cirrhosis, control subjects and patients with extrahepatic biliary tract obstruction following the intravenous administration of 5-hydroxytryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. The recovery of administered 5-hydroxytryptophan as urinary 5-hydroxyindoleacetic acid was significantly greater in patients with cirrhosis than that observed in control subjects or patients with biliary tract obstruction.

2. The administration of excesses of sodium orthoaminobenzoate together with 5-hydroxytryptophan or 5-hydroxytryptamine to rats resulted in a significantly greater urinary excretion of 5-hydroxyindoleacetic acid than was observed when these compounds were administered together with sodium chloride.

3. The data indirectly support the concept that 5-hydroxytryptamine conjugation may occur in the liver and suggest that in the presence of excesses of 5-hydroxytryptophan, 5-hydroxytryptamine conjugation may be impaired in hepatic cirrhosis.

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THE KINETICS OF BICARBONATE REABSORPTION DURING ACUTE RESPIRATORY ACIDOSIS*

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It is well-known that acute elevation of blood pCO_2 is accompanied by a rise in the renal reabsorption of bicarbonate (1–3). The exact mechanism through which this occurs has not been established, but it is thought to result from accelerated tubular hydrogen secretion due either to the lowering of cellular pH or to an increased formation of carbonic acid.

The present experiments were undertaken in an attempt to define further the nature of the reabsorptive process during acute respiratory acidosis. The data demonstrate that during an acute sustained increase of carbon dioxide tension, progressive elevation of plasma bicarbonate concentration was accompanied by a curvilinear increase in the rate of bicarbonate reabsorption. The relationship between plasma bicarbonate concentration and the rate of reabsorption was found, by means of the double reciprocal plot, to take the form $y = \frac{ax}{b + x}$. The possible significance of this finding has been considered.

MATERIALS AND METHODS

Twelve experiments were performed on female mongrel dogs anesthetized with sodium pentobarbital. An endotracheal tube fitted with an inflatable balloon was introduced into the trachea and connected either to a calibrated volume displacement ventilator (Etsten Ventilator) (4) or to a Mine Safety "Pneophore." Respiratory movements were inhibited by the administration of gallamine triethiodide (Flaxedil®) so that respiratory exchange could be controlled by the ventilator. Blood pCO_2 was increased to values of approximately 90 to 100 mm. Hg by having the animal breathe a mixture of 9 per cent CO_2 and 91 per cent oxygen. This tension was then kept as constant as possible. When elevated blood pCO_2 had been steadily maintained for at least one hour and adequate urine flow (3.6 to 6.2 ml. per minute) had been achieved by constant infusion of isotonic mannitol, plasma bicarbonate concentration was progressively increased by intravenous infusion of 0.142 to 0.285 molar solution of sodium bicarbonate at a rate of 7 ml. per minute. In the majority of the experiments, it was necessary to increase ventilation slightly in order to prevent the rise in CO_2 tension which usually followed the rapid infusion of bicarbonate.

The clearance of exogenous creatinine was used as a measure of glomerular filtration rate. Urine was collected under mineral oil through an inlying catheter and the bladder was emptied by manual compression at the beginning and at the end of each 10 minute collection period. Heparinized blood samples were drawn anaerobically from the femoral artery. Plasma and urine were analyzed for creatinine by a modification of the method of Bonsnes and Taussky (5) and for total CO_2 by the manometric method of Van Slyke. Blood and urine pH were measured anaerobically at 37° C. using a Cambridge Research Model pH meter. Concentration of bicarbonate and dissolved CO_2 were calculated from the Henderson-Hasselbalch equation, using a pK′ for carbonic acid of 6.1 and 0.0591 α equal to 0.0301 for plasma and 0.0309 for urine. Filtered bicarbonate was taken as the product of the filtration rate and the plasma bicarbonate concentration (corrected for a Donnan factor of 1.05). Potassium in serum was determined with an internal standard flame photometer.

Seven control experiments were performed with the animals breathing 100 per cent oxygen and with the pCO_2 maintained at normal levels by controlled ventilation with the Etsten Ventilator. Plasma bicarbonate concentration was increased by intravenous infusion of 0.142 to 0.595 molar solution of sodium bicarbonate at a rate of 7 ml. per minute. The higher concentrations of bicarbonate used in the control studies were necessary in order to achieve plasma bicarbonate concentrations comparable to those attained in the high pCO_2 experiments.

RESULTS

Table I shows the data from a typical experiment in which plasma bicarbonate level was raised progressively during acute, sustained respiratory acidosis. Initially, when plasma bicarbonate was 23.5 mEq. per L., there was only slight excretion of bicarbonate and thus nearly complete reabsorption. As the plasma level was

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TABLE I

*Effect of acute sustained respiratory acidosis on bicarbonate reabsorption during progressive elevation of plasma bicarbonate levels**

Time	Plasma		GFR	Urine flow	HCO_3^-		
	pCO_2	HCO_3			Filt.	Excr.	Reabs.
min.	*mm. Hg*	*mEq./L.*	*ml./min.*	*ml./min.*	*mEq./min.*	*μEq./min.*	*mEq./100 ml. GF*
Dog No. 4, 11.8 Kg.							
−105	Dog breathing 9% CO_2, 91% O_2						
−70	Mannitol, 50 Gm./L. I.V. at 7 ml./min.						
0–10	97	23.5	33.0	4.6	0.78	17	2.31
10–20	93	23.5	34.1	5.3	0.80	23	2.28
22	$NaHCO_3$, 0.143 M; mannitol, 25 Gm./L. I.V. at 7 ml./min.						
25–35	88	23.5	35.3	6.2	0.83	31	2.26
35–45	95	31.1	35.9	6.2	1.12	82	2.89
45–55	94	34.2	36.6	6.5	1.25	125	3.07
55–65	96	34.9	33.3	6.1	1.16	135	3.08
65–75	94	36.9	34.8	6.3	1.28	169	3.19
75–85	92	39.1	33.3	6.3	1.30	203	3.29
87	$NaHCO_3$, 0.214 M at 7 ml./min.						
90–100	95	41.2	33.9	6.5	1.40	239	3.42
100–110	92	42.9	32.3	6.5	1.39	289	3.41
110–120	89	45.5	33.1	6.7	1.51	356	3.49
120–130	92	48.1	33.0	6.9	1.59	413	3.57
130–140	96	51.6	31.0	6.7	1.60	453	3.70
140–150	99	54.3	37.2	7.9	2.02	613	3.78

* Plasma bicarbonate values have been corrected for a Donnan factor of 1.05.

elevated, there was a prompt and progressive increase in both the excretion and reabsorption of bicarbonate. The reabsorptive rate observed at 54.3 mEq. per L., the highest plasma level studied, was 3.78 mEq. per 100 ml. of glomerular filtrate. Plasma carbon dioxide tension ranged between 88 and 99 mm. Hg during the course of the study.

Table II shows the results of another representative experiment. The data again demonstrate a progressive increase in both the excretion and reabsorption of bicarbonate as the plasma bicarbonate concentration was elevated from 28.8 to a final value of 55.8 mEq. per L. The final reabsorption was 4.29 mEq. per 100 ml. of glomerular filtrate. As in the experiment shown in Table I, pCO_2 and glomerular filtration were nearly constant during the course of the study.

The results of all 12 experiments are shown in Figure 1. On the abscissa is plasma (filtrate)

TABLE II

*Effect of acute sustained respiratory acidosis on bicarbonate reabsorption during progressive elevation of plasma bicarbonate levels**

Time	Plasma		GFR	Urine flow	HCO_3^-		
	pCO_2	HCO_3			Filt.	Excr.	Reabs.
min.	*mm. Hg*	*mEq./L.*	*ml./min.*	*ml./min.*	*mEq./min.*	*μEq./min.*	*mEq./100 ml. GF*
Dog No. 8, 14.5 Kg.							
−100	Mannitol, 50 Gm./L. I.V. at 7 ml./min.						
−95	Dog breathing 9% CO_2, 91% O_2						
0–10	82	28.8	49.0	4.4	1.41	53	2.77
10–20	82	28.7	46.8	4.4	1.34	48	2.76
22	$NaHCO_3$, 0.179 M; mannitol, 25 Gm./L. I.V. at 7 ml./min.						
25–35	87	33.9	49.6	4.8	1.68	80	3.23
35–45	95	36.8	46.4	4.8	1.71	148	3.37
45–55	94	43.4	47.4	5.6	2.06	334	3.64
55–65	94	44.6	46.3	5.7	2.07	319	3.78
67	$NaHCO_3$, 0.262 M at 7 ml./min.						
70–80	93	51.7	45.5	6.6	2.35	469	4.13
80–90	92	54.1	45.1	6.7	2.44	532	4.23
90–100	91	55.8	44.2	6.6	2.47	576	4.29

* Plasma bicarbonate values have been corrected for a Donnan factor of 1.05.

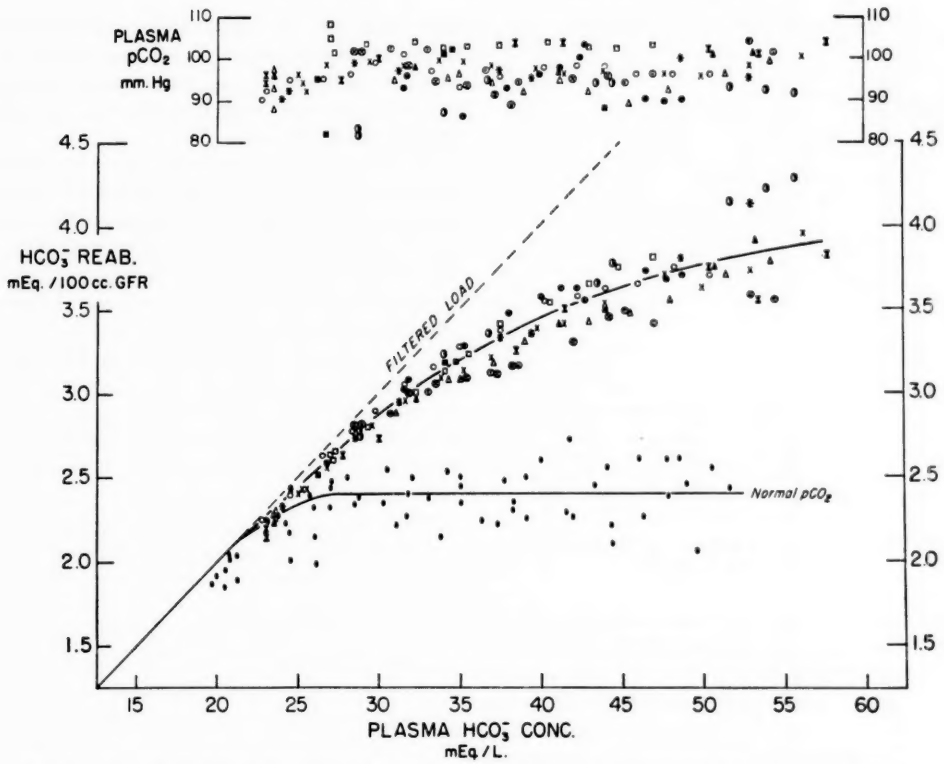

FIG. 1. EFFECT OF ACUTE SUSTAINED RESPIRATORY ACIDOSIS ON BICARBONATE REABSORPTION DURING PROGRESSIVE ELEVATION OF PLASMA BICARBONATE CONCENTRATION (12 EXPERIMENTS)

The curved line has been drawn through the experimental points by inspection. Data from the seven control experiments, carried out at normal pCO_2 values, are shown in the lower half of the chart. Values for pCO_2 of this group (not shown) ranged between 35 and 45 mm. of Hg.

bicarbonate level (mEq. per L.) and on the ordinate is bicarbonate reabsorption (mEq. per 100 ml. of glomerular filtrate). The straight line bisecting the chart indicates the filtered load of bicarbonate. The curved line was drawn through the experimental points by inspection. It can be seen that with elevation of the plasma bicarbonate level from 24 to 55 mEq. per L. there was a curvilinear rise in bicarbonate reabsorption, which, at the highest plasma concentration studied, reached a mean value of approximately 3.8 mEq. per 100 ml. of glomerular filtrate. Frank excretion of bicarbonate began at a plasma level of 26 to 30 mEq. per L. The observed values for plasma pCO_2 shown in the upper part of Figure 1 ranged from 82 to 108 mm. Hg. In eight of the 12 experiments, however, the pCO_2 remained within a range of 10 mm. Hg and in the group as a whole pCO_2 showed no significant trend during the course of the study. Variations in glomerular filtration rate during the course of the experiments were usually small and in no instance differed from the control values by more than 20 per cent.

In contrast, during the seven control experiments (Figure 1), bicarbonate reabsorption remained at approximately 2.4 mEq. per 100 ml. of glomerular filtrate for the group as a whole when plasma bicarbonate concentration was progressively elevated from normal levels to more than 50 mEq. per L. The range of reabsorptive values was 2.2 to 2.7 mEq. per 100 ml. of glomerular filtrate. pCO_2 remained between 35 and 45 mm. Hg throughout the study; these values are not shown in Figure 1.

Serum potassium concentration fell in both the

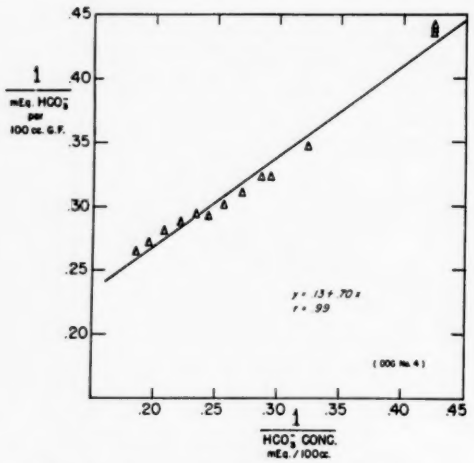

FIG. 2. DOUBLE RECIPROCAL PLOT OF DATA FROM TABLE I

The line through the points was calculated by the method of least squares.

control and the respiratory acidosis studies. The final mean value in the control group was 2.1 mEq. per L. (range, 1.7 to 2.4). S.E.$_{\bar{x}}$ equaled 0.12. In the acidosis group the value was 2.3 mEq. per L. (range, 1.7 to 2.9). S.E.$_{\bar{x}}$ equaled 0.13. The difference between the means was not significant ($p > 0.2$).

DISCUSSION

It has previously been shown that acute elevation of plasma bicarbonate concentration increases the renal reabsorption of bicarbonate (1–3). The present data demonstrate that the reabsorptive rate is a function of the plasma bicarbonate level, and that elevation of pCO_2 does not simply increase the reabsorptive capacity to a single new constant rate. Frank bicarbonate excretion began at a plasma level of approximately 26 to 30 mEq. per L., and there was a progressive increase in both the reabsorption and excretion of bicarbonate as the plasma concentration was elevated. Over the range of 26 to 55 mEq. per L. in the plasma there was a curvilinear rise in bicarbonate reabsorption which reached a final average value of 3.8 mEq. per 100 ml. of glomerular filtrate. The failure of previous workers to note such a curvilinear rise in bicarbonate reabsorption during acute respiratory acidosis (3) may have resulted from the fact that relatively few observations were made between plasma bicarbonate concentrations of 26 and 40 mEq. per L., a range over which the increasing rates of both reabsorption and excretion are most apparent.

In an attempt to find a simple mathematical function which might describe the curvilinear relationship between plasma bicarbonate concentration and the rate of bicarbonate reabsorption, the reciprocals of the values of the two variables were plotted one against the other. Figures 2 and 3 show the data from Tables I and II plotted in this fashion. The lines drawn through the points were calculated by the method of least squares. In each instance the r value was 0.99 ($p < 0.01$). In all 12 experiments the values for r were found to lie between 0.97 and 0.99 ($p < 0.01$).

From the linearity of the double reciprocal plot it follows that the relationship between plasma bicarbonate concentration and the rate of reabsorption takes the form $y = \frac{ax}{b + x}$, a rectangular hyperbola.[1] There is no obvious explanation for this finding, but two considerations suggest a possible working hypothesis. First, previous observations on the effects of acetazolamide in metabolic acidosis suggest that carbonic anhydrase plays a critical role in the reabsorption of most, if not all, filtered bicarbonate (6). Second, the relationship between plasma concentration and rate of reabsorption in the present study takes the same form as the equation which describes the initial velocity of an enzymatic reaction as a function of substrate concentration $\left[v = \frac{V_{max}\, S}{Km + S} \text{ (Michaelis-Menten)}\right]$. As a tentative interpretation, it is therefore proposed that during acute respiratory acidosis carbonic anhydrase becomes rate-limiting in the overall process of bicarbonate reabsorption. It is recognized that data consistent with the kinetic theory, while providing presumptive evidence

[1] This statement applies only at plasma concentrations above 23 to 25 mEq. per L. At plasma bicarbonate concentrations below this level, reabsorption of bicarbonate is known to be virtually complete and the relationship between the two variables is thus $y = x$. It would thus appear that there are two distinct components to the reabsorptive pattern observed between 0 and 55 mEq. per L.

in its support, can in no way be taken as proof of the hypothesis.

In the present formulation the bicarbonate concentration of the initial glomerular filtrate has been used as the expression of substrate concentration. No assumptions were made concerning changes in bicarbonate concentration during the passage of filtrate along the renal tubule. However, the hypothesis carries the implication that either: *1*) The bicarbonate concentration remains relatively constant over all but an insignificant segment of the renal tubule, or that *2*) if major changes in concentration occur, they bear such a relationship to the initial concentration that this latter value may still be taken as equivalent to substrate in the Michaelis-Menten equation. The apparent velocity constant (Km) for this system is 6.0 mEq. per 100 ml. of filtrate (S.E. $\pm$ 0.6) and the apparent V_{max} is 8.4 mEq. per 100 ml. of filtrate (S.E. $\pm$ 0.6).

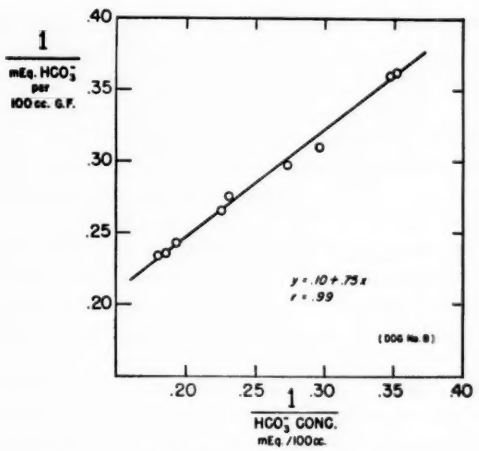

FIG. 3. DOUBLE RECIPROCAL PLOT OF DATA FROM TABLE II

The line through the points was calculated by the method of least squares.

It is noteworthy that the pattern of bicarbonate reabsorption in acute respiratory acidosis at plasma levels above 26 mEq. per L. is similar to the one found previously after the administration of acetazolamide to animals with metabolic acidosis (6). Acetazolamide is known to inhibit carbonic anhydrase *in vitro*, and it was suggested that the rate of reabsorption following injection of graded quantities of this drug was a function of residual enzyme activity in the renal tubular cells. The data from these studies, when tested by means of the double reciprocal plot, were also found to take the form of $y = \frac{ax}{b + x}$. Thus, as in the present study, the overall process of reabsorption appeared to follow the Michaelis-Menten relationship. It was tentatively suggested that at plasma bicarbonate levels below the normal threshold, partial inhibition of carbonic anhydrase makes enzyme activity rate-limiting in the process of bicarbonate reabsorption (6).

The virtually complete reabsorption of bicarbonate in the absence of inhibitor suggests that the enzymatic reaction is not the rate-limiting step in the normal at subthreshold plasma levels (6). With a low concentration of bicarbonate in the filtrate it seems likely that the rate of filtration of bicarbonate is limiting and that nearly complete removal of bicarbonate takes place before substrate comes into contact with the enzyme in the more distal tubular segments. As plasma bicarbonate rises, more enzyme would be expected to enter into the reaction as substantial quantities of bicarbonate reach additional segments. According to this view, enzyme is present in excess until a filtrate concentration of approximately 26 mEq. per L. is reached. At this point carbonic anhydrase all along the tubule is finally involved in the reabsorptive process and frank bicarbonate excretion begins. In the normal, regardless of further elevation of plasma bicarbonate concentration, the reabsorptive rate remains constant at approximately 2.4 mEq. per 100 ml. of glomerular filtrate. It is conceivable that a normal pCO_2 imposes this limit by preventing full utilization of enzyme activity. In acute respiratory acidosis this limit is apparently removed and, according to the present hypothesis, the enzymatic step becomes rate-limiting in the reabsorptive process.

The following schema is suggested as a possible explanation for the experimental findings in terms of the present hypothesis. Extrusion of hydrogen ions from the cell releases hydroxyl ions which are neutralized by carbon dioxide under the catalytic activity of carbonic anhy-

drase ($OH^- + CO_2 \overset{CA}{\rightleftharpoons} HCO_3^-$).[2] Carbon dioxide tension is assumed to influence hydrogen ion excretion independently of the enzymatic reaction. Thus, two rate-limiting steps can be visualized: *1*) hydrogen ion secretion and *2*) the enzymatic reaction which involves carbonic anhydrase. With normal pCO_2, hydrogen ion secretion is fixed at a constant maximal rate. The concomitant hydroxyl ion production in the cell occurs at a rate so slow that carbonic anhydrase is not the rate-limiting factor in the reaction by which the hydroxyl ion is buffered with carbon dioxide. Such a fixed rate of hydrogen ion secretion would account for the constant maximum rate of bicarbonate reabsorption which is observed at plasma bicarbonate concentrations above 26 mEq. per L. In contrast it is postulated that elevation of pCO_2 removes the limit on hydrogen ion secretion so that the latter increases as bicarbonate concentration in the filtrate is progressively elevated above 26 mEq. per L. and as the pH of the filtrate rises. With concomitant increases in hydroxyl ion production, carbonic anhydrase becomes rate-limiting and the reaction follows the Michaelis-Menten relationship.

According to this schema, the hydroxyl ion is the limiting substrate in the reaction. However, since the data appear to follow the Michaelis-Menten relationship when plasma bicarbonate concentration is plotted as substrate, it would follow as a corollary that intracellular hydroxyl ion concentration bears a direct relationship to the bicarbonate level in the filtrate.

The present data seem clearly to demonstrate that bicarbonate reabsorption rises in a curvilinear fashion as a function of plasma bicarbonate concentration but it is recognized that the theoretic formulation is highly tentative. It is hoped, however, that this framework may serve as a useful basis for further investigation.

[2] This sequence of events, which has been suggested as the mechanism for gastric acid secretion (7) and for urinary acidification (8–10), assumes that secreted hydrogen ions are derived ultimately from water. Although the reaction is bimolecular, since carbon dioxide is always in excess relative to the hydroxyl ion, the kinetics are those of a monomolecular reaction.

SUMMARY

Studies of renal bicarbonate reabsorption in acute respiratory acidosis have demonstrated a curvilinear rise in reabsorption as plasma bicarbonate concentration was progressively elevated from 24 to 55 mEq. per L. The average final value for bicarbonate reabsorption was 3.8 mEq. per 100 ml. of glomerular filtrate. Frank excretion of bicarbonate began at a plasma level of approximately 26 to 30 mEq. per L. The relationship between plasma bicarbonate concentration and the rate of bicarbonate reabsorption has been found to have the form $y = \frac{ax}{b + x}$. As a tentative explanation for these findings it has been suggested that during acute respiratory acidosis an enzymatic reaction involving carbonic anhydrase is rate-limiting in the process of bicarbonate reabsorption.

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MUCOPOLYSACCHARIDE CONTENT OF SKIN IN PATIENTS WITH PRETIBIAL MYXEDEMA *†

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It is well-known that the skin of patients with primary and secondary myxedema contains a mucinous infiltrate. This extracellular deposition of metachromatically staining material has been interpreted as being a complex of acid mucopolysaccharide and protein (1). It has not been defined chemically. The infiltrate disappears from the skin of patients with generalized myxedema who are given 120 to 180 mg. of desiccated thyroid daily for six to eight weeks (1). A deposit, histochemically like that which occurs in generalized myxedema, occasionally presents locally in the pretibial skin of patients with thyrotoxicosis and exophthalmos (2, 3). When the patient with localized pretibial myxedema is given 180 mg. of desiccated thyroid a day, however, no clinical or histochemical change occurs in the myxedematous plaque.

In 1947 Watson and Pearce isolated a crude mucopolysaccharide fraction from the pretibial plaques of two patients with pretibial myxedema. They found considerably less of this material in skin from the amputated leg of an elderly man (4). We have found no record of the comparison of chemical or histochemical findings of myxedematous areas to other areas of the skin of patients with pretibial myxedema, or comparison with control patients of similar age.

We wish to report a chemical study of the mucopolysaccharide concentration of the skin of six euthyroid patients with pretibial myxedema and exophthalmos and eight patients of comparable sex distribution and mean age without thyroid disease. The acid mucopolysaccharide content of the myxedematous areas was found to be considerably elevated when compared to clinically uninvolved skin from the same patients. The clinically uninvolved areas were found to contain a moderately increased concentration of acid mucopolysaccharide when compared to normal skin from patients without thyroid disease.

METHOD

Under local anesthesia skin biopsies were taken from four women and two men, 27 to 58 years of age, with extensive pretibial myxedema associated with exophthalmos manifested by increased exophthalmometer measurements or by serious collateral eye signs. Biopsies were obtained from the affected pretibial skin and an area of clinically uninvolved skin of the upper thigh and occasionally from the deltoid area of the shoulder. The clinical story and histology of previous skin biopsies of three of these patients were summarized in 1954 (2).

Skin biopsies were also obtained on six females and two males, six and one-half to 78 years of age, who had no demonstrable thyroid disease or pretibial myxedema, past or present. These biopsies were obtained from the skin of the leg at the time of orthopedic surgery.

Details of the method of assay of acid mucopolysaccharide used in this study have been published by Bollet (5). Values are expressed as micrograms of uronic acid in the acid mucopolysaccharide fraction per 100 mg. of dried, fat free tissue; carbazole and orcinol methods of determination were used. This procedure was demonstrated to have a replicate reproducibility of approximately ± 7 per cent.

RESULTS

The acid mucopolysaccharide concentrations of the clinically myxedematous pretibial skin are presented in Table I, A. The mean value was 990 μg. of uronic acid per 100 mg. of tissue using the carbazole method, and 735 μg. using orcinol. The mean values for the clinically uninvolved areas of skin from the same individuals were 171 and 146 μg. per 100 mg. of tissue, respectively

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‡ John and Mary Markle Scholar in Medical Science.

TABLE I

Uronic acid content of the acid mucopolysaccharide fraction of skin biopsies of patients with pretibial myxedema, using carbazole and orcinol methods of determinations *

Patient	Carbazole	Orcinol	Ratio of carbazole to orcinol values
A. Myxedematous areas (pretibial)			
1	1,770	1,733	1.0
2	2,010	1,524	1.3
3	804	519	1.6
4	408	146	2.8
5	440	298	1.5
6	507	298	1.7
Mean	990	753	1.7
B. "Normal" areas			
1 (thigh)	98.7	126	0.8
2 (thigh)	221	180	1.2
(shoulder)	303	183	1.3
3 (shoulder)	108	95.5	1.1
4 (thigh)	145	141	1.0
5 (thigh)	184	164	1.1
6 (thigh)	138	129	1.2
Mean	171	146	1.1

* Results are expressed as micrograms of uronic acid per 100 mg. of dried, fat free skin.

(Table I, B). The latter values were higher than the mean values found in the normal skin of eight individuals, which were 89 and 98 μg. per 100 mg., respectively (Table II). The differences in carbazole values from clinically uninvolved skin of patients with pretibial myxedema as compared to skin from control patients without thyroid disease are significant, with a p value of < 0.01.

TABLE II

Uronic acid content of the mucopolysaccharide fraction of biopsies of skin of subjects without thyroid disease, using carbazole and orcinol methods of determination *

Patient	Carbazole	Orcinol	Ratio of carbazole to orcinol values
1 (shin)	95.2	125	0.8
2 (shin)	93.0	97	1.0
3 (shin)	64.6	81.4	0.8
4 (thigh)	78.6	115	0.7
5 (shin)	90.0	60.5	1.5
6 (thigh)	67.8	70.7	1.0
7 (thigh)	104	115	0.9
8 (shin)	115	115	1.0
Mean	89	98	1.0

* Results are expressed as micrograms of uronic acid per 100 mg. of dried, fat free skin.

Other variables that might affect these data were examined. The mean age of the normal controls was 48 years as compared to a mean age of 45 years in patients with thyroid disease. No correlation was found between age and acid mucopolysaccharide value in patients within either group.

Table III presents a comparison of some clinical features with the acid mucopolysaccharide concentration found in the areas of pretibial myxedema by the carbazole method.

Mucopolysaccharide levels were lower in the three patients with the lesions of longest duration. The areas of pretibial myxedema in these patients were observed to be diminishing. In addition, these patients had been receiving desiccated thyroid for at least four years. It should be noted, however, that Patients 1 and 3 had also been on desiccated thyroid from the onset of pretibial myxedema until a few weeks before biopsy. Exophthalmometer measurements were higher in patients with thyroid disease of longer duration. It is of particular interest that Patients 1 and 3, the two patients with least protrusion of the eyes, were the only pretibial myxedema patients in whom values for acid mucopolysaccharide in skin of thigh overlapped mucopolysaccharide values in leg skin from patients without thyroid disease. The acid mucopolysaccharide concentration did not seem to be related to the history of surgery or radioactive iodine treatment for relief of thyrotoxicosis.

TABLE III

A comparison of some clinical features of patients with pretibial myxedema

Duration of pretibial myxedema	Carbazole value	Exophthalmometer measurements at the time of biopsy	History of thyroid disease*
years	*μg./100 mg.*	*mm.*	
0.33	1,770	16.5/17.5	Th., Tx., Eu., T.
1.8	2,010	21.5/20.5	Th., I^{131}, Eu.
2.5	804	17/18	Th., Tx., Eu., T.
4.0	408	24/24	Th., Tx., on thyroid
10.0	440	24.5/27.5	Th., I^{131}, on thyroid
13.0	507	23/24	Th., Tx., on thyroid

* Th., thyrotoxicosis; Tx., surgical thyroidectomy; I^{131}, I^{131} thyroidectomy; Eu., euthyroid; and T., previously on thyroid medication.

Higher acid mucopolysaccharide levels in association with pretibial myxedema were accompanied by a higher mean ratio of carbazole to orcinol values.

DISCUSSION

Curtis, Cawley and Johnwick in 1949 reported the association of malignant exophthalmos and pretibial myxedema and suggested that both the malignant exophthalmos and the localized myxedema might be caused by a local effect of pituitary thyroid stimulating hormone (TSH) on the extraocular tissues and on the corium of the skin of the lower leg (6). We have demonstrated that the administration of TSH to dogs results in increase in concentration of acid mucopolysaccharide in the dog thyroid gland (7). The generalized increase in the concentration of acid mucopolysaccharide in the skin of patients with pretibial myxedema might also result from the same stimulus. Differences in concentration might be explained by local variations in tissue responsiveness and to physical factors such as gravity and tissue pressure. The increased concentration of acid mucopolysaccharide demonstrated histochemically in the retro-orbital tissues of coincident exophthalmos is presumably part of this response (3). Since pretibial myxedema can occur with either hypo- or hyperthyroidism (2), and since our patients were euthyroid at the time biopsies were obtained, it seems unlikely that lack of thyroid hormone is the causative factor of this condition, although evidence would seem to indicate that it is the causative factor in generalized myxedema. The observation that localized myxedema in one patient with coincident exophthalmos disappeared completely with the onset of pituitary failure after pituitary surgery is evidence for a possible role of TSH in the etiology of localized myxedema (8). These two states with similar histochemical findings thus appear to differ in some important aspects.

The increase in the carbazole to orcinol ratio found in the myxedematous areas suggests a qualitative change in the mucopolysaccharide of the skin. The ratio of 1.7 to 1 found approaches the 2 to 1 ratio characteristic of heparitin sulfate (5), and suggests a predominant increase in this type of polysaccharide.

The finding that the changes in mucopolysaccharide content of the skin are not limited to the pretibial area indicates that the term "localized pretibial myxedema" may be clinically correct, but is chemically incorrect.

SUMMARY

A chemical assay of the skin of six patients with "localized" pretibial myxedema was made. All six patients had exophthalmos and were euthyroid at the time the skin biopsies were made. Biopsies were made of the clinically involved skin of the shin and of clinically uninvolved skin of the upper leg and the deltoid area. The acid mucopolysaccharide concentration of these skin specimens was compared with the concentration found in the leg skin biopsies obtained during orthopedic operations on eight comparable individuals who had never had thyroid disease.

The mean value for acid mucopolysaccharide concentration in the clinically myxedematous pretibial skin was 990 μg. of uronic acid per 100 mg. of tissue using the carbazole method, and 753 μg. using orcinol. The mean values for the clinically uninvolved areas of skin from the same individuals were 171 and 146 μg. per 100 mg. of tissue, respectively. The latter values are higher than the mean values found in the normal skin of eight individuals without thyroid disease, which were 89 and 98 μg. per 100 mg., respectively.

Higher acid mucopolysaccharide values found in pretibial myxedema were associated with a higher mean ratio of carbazole to orcinol values.

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TISSUE AND RENAL RESPONSE TO CHRONIC RESPIRATORY ACIDOSIS *

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The elevation of pCO_2 which occurs during respiratory acidosis is accompanied by a rise in the concentration of bicarbonate and a fall in the concentration of chloride in serum (1). The increased bicarbonate concentration results from both cellular and renal responses. The cellular response is characterized by the exchange of extracellular hydrogen ions for intracellular cations, thereby contributing to the elevation of the extracellular concentration of bicarbonate (2, 3).

The renal factors responsible for the elevated bicarbonate concentration may be divided into two phases: *1*) acid excretion, and *2*) bicarbonate reabsorption. In acute human experiments, augmented acid excretion, as evidenced by increased excretion of ammonia and titratable acid, has been reported (4). Barker, Singer, Elkinton and Clark observed these changes also, but noted that if the urine was acid prior to breathing carbon dioxide, further increase in acid excretion may not occur (5). Perhaps this explains, in part at least, the failure of Longson and Mills to find increased acid excretion during short term human experiments (6).

In acute respiratory acidosis tubular reabsorption of bicarbonate is increased (7). Moreover, in chronic respiratory acidosis in dogs, there is a further augmentation of reabsorption of bicarbonate that varies directly with the duration of the imposed acidosis (8).

The fall in the concentration of serum chloride during respiratory acidosis may also result from both cellular and renal responses. The only cell into which chloride has been clearly demonstrated to enter during respiratory acidosis is the red cell (1, 3, 9). The role of renal losses in the production of hypochloremia is less certain. In short term human experiments, chloruresis has not been observed during respiratory acidosis (5, 10). However, in the rat, Epstein, Branscome and Levitin observed increased chloride excretion during the first 24 hours of an imposed respiratory acidosis (11).

The present study in the rat was undertaken in an attempt to delineate the pattern of renal and extrarenal responses to respiratory acidosis. The excretion of sodium, potassium and chloride was studied during the early and late phases. The contribution of acid loss, as ammonia and titratable acid, to the elevation of plasma bicarbonate concentration was examined. The activities of the renal enzymes concerned with acid excretion, glutaminase and carbonic anhydrase, were determined in order to ascertain whether adaptation of these enzymes occurs as a result of chronic respiratory acidosis. Finally, possible extrarenal contribution to the buffering of carbon dioxide was examined in both muscle and bone.

PROCEDURE

Male Sprague-Dawley rats weighing between 300 and 400 Gm. were used in all experiments. In early experiments, the rats were tube-fed a liquid diet twice daily that provided a total of 2.0 Gm. of protein, 1.5 Gm. of fat and 2.0 Gm. of glucose per day together with essential minerals and vitamins. Later experiments used the same diet; however, the diet was offered in a standard drinking fountain, a measured amount of diet being offered twice daily. Particular care was taken to ascertain that each rat took the entire amount of diet offered. The intake of equal amounts of diet by both control and experimental animals is essential, for, as has been previously pointed out, variations in dietary intake result in altered excretion of acid by the kidneys (12). With respect to sodium, potassium and chloride, two different diets were used. The standard diet (Std. Diet) provided 2.0 mEq. of sodium, 1.0 mEq. of potassium and 3.0 mEq. of chloride per day. The electrolyte deficient diet (E.D.

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Diet) contained only negligible amounts of these ions. All rats were given distilled water *ad libitum*.

A large box was constructed for use as a metabolic cage for those animals subjected to respiratory acidosis. The design was such that daily urines could be collected from individual rats. The proper content of carbon dioxide within the box was maintained by automatic electrical controls which intermittently released carbon dioxide into the box. By this method, the carbon dioxide content of the box could consistently be maintained within ± 1.0 per cent of the desired value. Oxygen content of the atmosphere in the box was frequently checked with an Orsat gas analysis apparatus, and was found to range from 19 to 21 per cent. It was not necessary to remove rats from the box during an experiment; feeding and cage cleaning were carried out through airtight arm ports. By means of air-conditioning, both control and experimental animals were kept in an atmosphere maintained nearly constant at 73° F.

Twenty-four hour urines were collected from individual rats under oil and preserved with phenylmercuric nitrate and toluene. Approximately midway through this study, changes in the urine collection system were made that resulted in different values for urinary constituents. The change was the application of a silicone coating to the surface of all glassware used in the collection system. This gave rise to an immediate flow of urine from the rat into the collection vessels and made it unnecessary to rinse the collection system at the end of each collection period, since there was no significant evaporation of urine on the siliconized surfaces. Using the new urine collection system, it was found that apparent ammonia excretion was approximately 1.66 times greater than that found using the old urine collection system. An additional control study, using the new urine collection system, was made in order to facilitate comparison with experimental groups. The study included eight groups of rats. Groups 1 through 3 were those in which the original urine collection method was used (Table II). The new urine collection method was used in the remaining five groups (Table II).

All groups were observed for a period of 11 days. Group 1 served as a control for Groups 2 and 3. In these three groups, all rats received the Std. Diet, and daily urines were collected for determination of pH, ammonia and titratable acidity. Group 2 was subjected to an atmosphere of 10 per cent carbon dioxide. Group 3 was subjected to 15 per cent carbon dioxide.

Group 4 served as a control for Groups 5 and 6. These three groups received the Std. Diet, and as in previous groups daily urines were collected for the determination of pH and acid excretion. In addition, sodium, potassium and chloride were determined in the urines from Groups 4 and 5. Group 5 was subjected to 10 per cent carbon dioxide. In an attempt to produce a marked additive effect on acid excretion, Group 6 was subjected to 10 per cent carbon dioxide for two days, then 15 per cent for two days, these cyclic changes being maintained throughout the experiment. These rats were sacrificed while breathing 10 per cent carbon dioxide. Groups 7 and 8 were a control group and a group subjected to 10 per cent carbon dioxide, respectively. In an attempt to magnify changes in plasma and muscle electrolytes, as well as in urinary excretion of electrolytes, these two groups received the E.D. Diet. Urines were collected and analyzed as in Groups 4 and 5.

METHODS

Rats subjected to carbon dioxide were killed while breathing an atmosphere having the same carbon dioxide content as was used for the experiment. This was accomplished by supplying an appropriate oxygen-carbon dioxide mixture to a small box into which the anesthetized rat's head was placed by way of a snugly fitting neck port. Both control and experimental animals were anesthetized by an intraperitoneal injection of pentobarbital. Blood was received anaerobically into a previously heparinized syringe from the abdominal aorta. Muscle samples were removed from the hind legs. In some animals, the bones of the hind legs were taken for electrolyte analysis. The kidneys were perfused with chilled isotonic saline in order to wash out all red blood cells, then removed and immediately frozen for enzyme assays.

Methods for the determination of plasma electrolytes, urine ammonia, pH and titratable acidity, muscle electrolytes and kidney glutaminase activity were those previously described from this laboratory (12, 13). Urine sodium and potassium concentrations were determined by flame photometry (14). Urine chloride concentration was determined by a modification of the Volhard-Harvey method (15). Whole blood pH was anaerobically determined at 37° C. by means of a Cambridge water-jacketed glass electrode and a Cambridge pH meter. Plasma carbon dioxide tension was estimated by means of the Henderson-Hasselbalch equation using the blood pH, plasma carbon dioxide content and a pK of 6.11. Buffer anion was estimated by means of a Singer-Hastings nomogram assuming a red cell hematocrit of 50 per cent in each case (16).

Electrolyte composition of marrow-free bone was determined in Groups 4 and 5. The method of analysis was a modification of the ion-exchange column method of Forbes and D'Ambruso (17).

Renal carbonic anhydrase activity was determined in water homogenates of kidney tissue. The method described by Ashby and Chan (18) was modified in the following way. A veronal-sodium veronal buffer (0.03 M) as suggested by Roughton and Booth (19) was substituted for the carbonate-bicarbonate buffer of the original method because the former provides a better pH range for the enzymatic reaction and does not adversely affect the enzyme. Since the reaction was carried out from pH 7.9 to pH 6.3, water soluble brom-thymol-blue was used as the indicator in a final concentration of 1.57 mg. per 100 ml. This concentration was below that demonstrated by Wilbur and Anderson to cause inhibition of the enzyme (20). The reaction was carried out at 3° C. (± 0.2° C.). The exact time for the reaction $CO_2 + H_2O$

TABLE I

Plasma electrolyte concentrations

Group	Procedure	Na	K	Cl	CO_2 content§	Whole blood pH§	pCO_2§	Buffer anion
		mEq./L.	*mEq./L.*	*mEq./L.*	*mEq./L.*		*mm. Hg*	*mEq./L.*
1 and 4*	Std. Diet only	†144.0 (10)‡ ±2.2	5.0 (10) ±.44	108.8 (10) ±2.8	24.7 (10) ±1.8	7.43 (5) ±.02	35.4 (5) ±2.6	48 (5) ±1.4
2 and 5*	Std. Diet + 10% CO_2	151.4 (10) ±3.9	4.73 (10) ±.46	91.8 (10) ±3.0	45.2 (10) ±2.3	7.28 (9) ±.04	102.3 (9) ±6.6	58 (9) ±4.0
3	Std. Diet + 15% CO_2	149.3 (4) ±3.1	4.31 (5) ±.25	85.3 (4) ±4.5	52.4 (4) ±3.2	7.15 (5) ±.07	147.7 (5) ±29.3	60 (5) ±1.3
6	Std. Diet + 10 and 15% CO_2	150.9 (4) ±4.0	3.60 (4) ±.34	93.5 (4) ±3.9	42.3 (4) ±2.9	7.19 (4) ±.01	108.3 (4) ±9.2	55 (4) ±2.2
7	E.D. Diet only	145.6 (5) ±1.8	4.81 (5) ±.42	104.6 (5) ±1.4	22.2 (5) ±1.9	7.39 (5) ±.01	36.7 (5) ±1.8	46 (5) ±1.4
8	E.D. Diet + 10% CO_2	156.4 (9) ±3.1	2.85 (9) ±.47	89.1 (9) ±2.4	46.2 (9) ±1.0	7.24 (9) ±.04	102.5 (9) ±2.7	58 (9) ±2.4

* Groups 1 and 4 both received the same Std. Diet. Methods for urine collection differed in the two groups. The electrolyte composition in blood was identical in both groups, and these data were pooled. Since the same circumstances existed for Groups 2 and 5, their data were also pooled.

† Mean and standard deviations.

‡ Number in parentheses, number of animals used.

§ The mean and standard deviation for pCO_2 was *not* estimated from the *mean* values for CO_2 content and pH. Instead, the pCO_2 was estimated for *each* rat from the CO_2 content and pH; then the mean and standard deviations were determined. As a consequence, there is an apparent disparity between the mean values for CO_2 content and pH on the one hand and pCO_2 on the other as these values appear in this table.

$= H_2CO_3$ to proceed sufficiently far to the right to lower the pH of the buffer to 6.3 without added enzyme was the control time. The amount of enzyme (kidney homogenate) necessary exactly to halve this time was defined as a unit of enzyme activity.

Throughout, results are reported as mean values for respective groups together with the standard deviation of the mean. P values were obtained from appropriate tables after the t value was calculated.

RESULTS

Rats used in these experiments were able to withstand an atmosphere of 10 per cent carbon dioxide for 11 days without obvious untoward effect. Experiments lasting 14 days gave rise to a mortality of approximately 5 per cent. An atmosphere of 15 per cent carbon dioxide resulted in a mortality of approximately 40 per cent within 11 days. A mortality of 100 per cent occurred within 30 hours when rats were subjected to 20 per cent carbon dioxide.

Degree of acidosis

Rats subjected to 10 or 15 per cent carbon dioxide atmosphere developed severe respiratory acidosis as evidenced by increased plasma CO_2 content, decreased blood pH and increased plasma pCO_2 (Table I). Fifteen per cent carbon dioxide gave rise to a more profound acidosis than did 10 per cent carbon dioxide in that the mean blood pH for Group 3 was significantly lower ($p < 0.01$) than those values found in Groups 2 and 5.

The somewhat higher CO_2 content found for Group 3 was, however, of only doubtful significance ($p > 0.02$) when compared with the CO_2 content of Groups 2 and 5. Although the mean blood pH of Group 6 (10 and 15 per cent carbon dioxide) was significantly lower ($p < 0.001$) than that value for Groups 2 and 5, the CO_2 content and pCO_2 were not significantly different. These data suggest that in both Group 3 and Group 6 the acidosis was more severe and the compensation less complete than in Groups 2 and 5.

There was no significant difference found in the degree of acidosis produced with 10 per cent carbon dioxide between groups that received the Std. Diet (Groups 2 and 5) and the E.D. Diet (Group 8) (Table I).

As was expected, respiratory acidosis produced

an elevation of whole blood buffer anion (16). Since the value found for 10 per cent carbon dioxide was not significantly different from that found for 15 per cent carbon dioxide (Table I), it would appear that under these experimental conditions, buffer anion concentration displayed a maximal concentration not entirely proportionately related to the degree of hypercapnea.

Plasma sodium, potassium and chloride

In all groups subjected to respiratory acidosis, the plasma sodium concentration was significantly increased above control values (Table I). Although acute respiratory acidosis has been demonstrated to accelerate the excretion of water (10), no increased urine volumes were observed in rats during chronic respiratory acidosis. The hypernatremia may have been the consequence of the inability of rats who are breathing rapidly to increase the intake of water sufficiently to replenish deficits resulting from enhanced respiratory water loss.

Plasma potassium concentrations were, in all groups with respiratory acidosis, significantly lower than control values (Table I). The only abnormally low potassium concentrations occurred in those rats receiving the potassium deficient E.D. Diet (Group 8). There was no definite relationship between plasma pCO_2 and potassium concentration (Table I). Potassium concentration and blood pH likewise had no definite relationship (Table I).

In all our experimental groups, the expected hypochloremia of respiratory acidosis was observed (Table I). The decrement in chloride concentration was, for the most part, only slightly less than the increment in carbon dioxide content of the plasma.

Acid excretion

Table II summarizes the cumulative acid excretion for experimental and control groups during the 11 days of observation. The actual differences between rats subjected to respiratory acidosis and control animals were small, although significant for the most part. Ammonia excretion was sig-

TABLE II

Cumulative acid excretion during respiratory acidosis for 11 days

Group (No. of rats)	Procedure	NH_3*	TA*	NH_3 + TA*
		mEq.	*mEq.*	*mEq.*
Group 1† (5)	Std. Diet only	14.0 ± 0.8	3.87 ± 0.52	17.71 ± 0.9
Group 2† (10)	Std. Diet + 10% CO_2	18.0 ± 2.4 p = < 0.001	4.05 ± 0.38 p = > 0.5	22.1 ± 2.9 p = 0.01
Group 3† (5)	Std. Diet + 15% CO_2	20.4 ± 2.2 p = < 0.001	4.09 ± 0.69 p = > 0.5	24.1 ± 2.8 p = < 0.01
Group 4‡ (5)	Std. Diet only	24.6 ± 0.9	3.26 ± 1.09	27.7 ± 1.6
Group 5‡ (4)	Std. Diet + 10% CO_2	26.2 ± 0.5 p = < 0.02 > 0.01	5.98 ± 1.04 p = 0.01	31.5 ± 0.8 p = < 0.01
Group 6‡ (5)	Std. Diet + 10 and 15% CO_2	28.9 ± 3.4 p = < 0.05 > 0.02	5.77 ± 0.84 p = < 0.01	32.9 ± 6.8 p = < 0.20 > 0.10
Group 7‡ (6)	E.D. Diet only	23.5 ± 1.0	4.59 ± 0.83	28.1 ± 1.7
Group 8‡ (10)	E.D. Diet + 10% CO_2	25.6 ± 2.5 p = < 0.001	4.40 ± 0.84	29.7 ± 3.0 p = < 0.20

* Cumulative excretion is expressed as mean and standard deviations. Statistical expressions compare experimental groups with their respective controls.

† Groups 1, 2 and 3, original urine collection method.

‡ Groups 4 through 8, later urine collection method.

nificantly increased in most of the groups subjected to either 10 or 15 per cent CO_2 (Groups 2, 3, 5 and 8), although in one instance the significance of the increase was borderline (Group 6). The cumulative excretion of titratable acid (TA) was increased in two groups (Groups 5 and 6). In all experimental groups excepting Group 8, the cumulative excretion of acid (NH_3 plus TA) rose significantly. The daily excretion of total acid

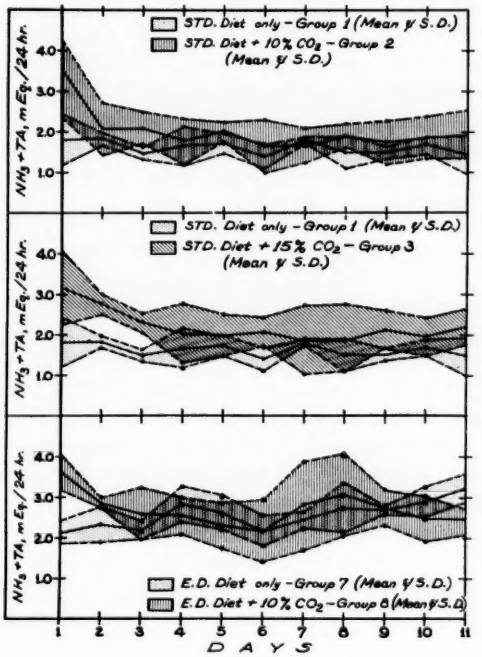
STD. Diet only - Group 1 (Mean ± S.D.)
STD. Diet + 10% CO2 - Group 2 (Mean ± S.D.)
STD. Diet only - Group 1 (Mean ± S.D.)
STD. Diet + 15% CO2 - Group 3 (Mean ± S.D.)
E.D. Diet only - Group 7 (Mean ± S.D.)
E.D. Diet + 10% CO2 - Group 8 (Mean ± S.D.)
NH3 + TA, m Eq./24 hr.
DAYS

FIG. 1. DAILY TOTAL ACID EXCRETION (NH_3 PLUS TA) DURING RESPIRATORY ACIDOSIS

for three representative groups (2, 3, 8) of rats subjected to respiratory acidosis is plotted in Figure 1. There was a tendency for the highest acid excretion to occur during the first two days of respiratory acidosis, after which acid excretion tended to fall to a level only slightly greater than that observed in control animals.

To examine the daily pattern of urine pH, NH_3 and titratable acid during respiratory acidosis, data from rats subjected to 10 per cent CO_2 are compared with their controls in Figure 2. All other groups behaved in similar fashion. The early rise in total acid excretion was the result of a rise in

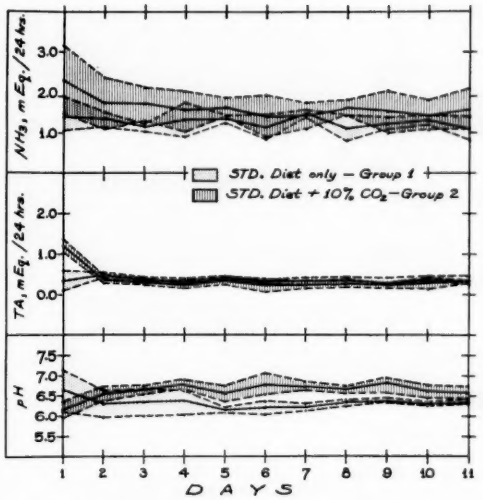

FIG. 2. MEAN DAILY URINARY ACID EXCRETION DURING RESPIRATORY ACIDOSIS

ammonia and titratable acid excretion. Ammonia excretion tended to remain slightly above control values throughout the study. Early in respiratory acidosis, urine pH tended to be lower than in the control groups but rose to control values or above, more or less paralleling the fall in ammonia excretion, as the study was continued.

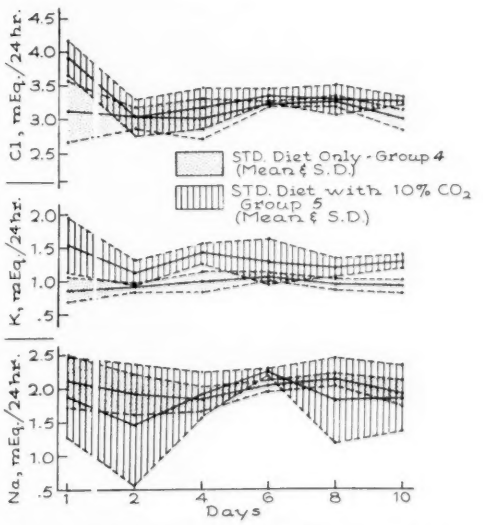

FIG. 3. MEAN DAILY URINARY EXCRETION OF NA, K AND CL DURING RESPIRATORY ACIDOSIS

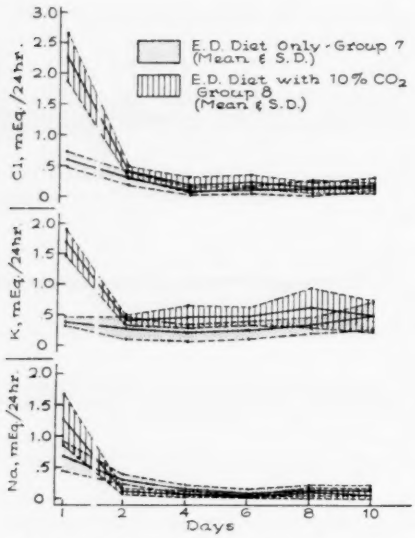

FIG. 4. MEAN DAILY URINARY EXCRETION OF NA, K AND CL DURING RESPIRATORY ACIDOSIS

Sodium, potassium and chloride excretion

The mean daily urinary excretion of sodium, potassium and chloride was compared in rats subjected to 10 per cent carbon dioxide while on Std. Diet (Figure 3) and while on E.D. Diet (Figure 4) with their respective controls. Sodium excretion was not significantly changed by respiratory acidosis. Both potassium and chloride excretion were significantly increased during the first day. Thereafter, chloride excretion fell to control values while potassium excretion remained slightly elevated throughout the study. Similar findings have been obtained by others (11). In Table III, the total cumulative mean excretions of sodium, potassium and chloride are given for the rats during respiratory acidosis on Std. and E.D. Diet. The excess potassium excreted in the groups with respiratory acidosis was strikingly similar for both dietary regimens (ΔK Std. Diet = 3.76 mEq. and ΔK E.D. Diet = 3.50 mEq.). To a lesser extent, this was true for chloride excretion (ΔCl Std. Diet = 2.65 mEq. and ΔCl E.D. Diet = 2.18 mEq.). This suggests that urinary potassium and chloride loss incident to a given degree of respiratory acidosis is essentially independent of the availability of these ions in the diet.

Muscle and bone electrolytes

Intracellular sodium of muscle was significantly decreased in all groups with respiratory acidosis as compared with control values (Table IV). This is in accord with previous findings in rats with chronic respiratory acidosis (23).

There was a slight lowering of muscle potassium in all groups subjected to respiratory acidosis, and this was significant in those groups given the Std. Diet and 10 or 15 per cent carbon dioxide (Table IV, Groups 2 and 5, and Group 3). Previously, high normal muscle potassium

TABLE III

Total cumulative mean excretion of Na, K and Cl

	Procedure	Sodium	Potassium	Chloride
		mEq.	*mEq.*	*mEq.*
Group 4	Std. Diet only (5)*	21.61† ±1.21	10.63 ±0.92	34.28 ±1.59
Group 5	Std. Diet + 10% CO_2 (4)	21.73 ±0.92	14.39 ±0.92 p = > 0.01 < 0.001	36.93 ±0.22 p = > 0.01 < 0.001
Group 7	E.D. Diet only (6)	2.35 ±0.51	3.27 ±1.38	2.28 ±0.27
Group 8	E.D. Diet + 10% CO_2 (5)	2.07 ±0.42 p = > 0.50 < 0.30	6.77 ±1.30 p = > 0.01 < 0.001	4.46 ±0.60 p = > 0.001

* Number in parentheses, number of animals used.
† Mean and standard deviation.

TABLE IV

Muscle and bone electrolytes

Group		1 and 4 (5)*	2 and 5 (5)	3 (5)	6 (5)	7 (5)	8 (5)
Procedure		Std. Diet only	Std. Diet + 10% CO_2	Std. Diet + 15% CO_2	Std. Diet + 10 and 15% CO_2	E.D. Diet only	E.D. Diet + 10% CO_2
Muscle†	Na_i‡	4.45§ ±0.43	3.31 ±0.53	3.16 ±0.78	2.82 ±0.45	4.26 ±0.42	2.86 ±0.54
	K	45.1 ±1.3	42.0 ±1.6	40.3 ±1.7	43.8 ±2.3	46.8 ±1.2	43.2 ±2.9
Bone‖	Na_i¶	254 ±13	258 ±8				
	K	16.6 ±1.6	20.0 ±2.1				
	Mg	578 ±13	480 ±21				
	Ca	13,070 ±100	13,320 ±100				

* Number in parentheses, number of animals used.
† mEq. per 100 Gm. fat-free dry muscle.
‡ Na_i in muscle was calculated from the total muscle sodium by correcting for the sodium in the extracellular fluid of the muscle sample after the method of Yannet and Darrow (21).
§ Group mean value and standard deviation.
‖ mEq. per Kg. fat-free dry bone.
¶ Na_i in bone was calculated from the total bone sodium by correcting for the sodium in the extracellular fluid of the bone sample. Extracellular fluid volume was derived by assuming all bone chloride to be extracellular (22).

values have been reported for rats with chronic respiratory acidosis (23).

In Table IV, electrolyte composition of bone during respiratory acidosis is compared with controls. The most striking finding was a marked fall in bone magnesium.

Renal glutaminase and carbonic anhydrase

Renal carbonic anhydrase activity failed to show any significant change after 11 days of respiratory acidosis (Table V). The increased bicarbonate reabsorption which occurred in the animals subjected to respiratory acidosis was not, therefore, mediated by adaptive increase in renal carbonic anhydrase activity as measured by the *in vitro* method employed.

Despite prolonged and persistent respiratory acidosis, the activity of renal glutaminase did not rise (Table V).

To explore the significance of chloride loads during respiratory acidosis, rats subjected to 10 per cent CO_2 were given in addition 3 mMole NH_4Cl daily and were killed after 11 days. The following results were obtained: plasma CO_2 content, 39.2 ± 2.4 mEq. per L.; plasma pCO_2, 90.9 ± 10.2 mm. Hg; plasma chloride, 93.6 ± 0.8 mEq. per L.; blood pH, 7.25 ± 0.08. Ammonia excretion was elevated by about 3 mEq. per day above those levels found in animals given 10 per

TABLE V

Renal glutaminase and carbonic anhydrase activities

Group	Procedure	Renal glutaminase*	Renal carbonic anhydrase†
1 and 4	Std. Diet only	‡608 (10)§ ±60	370 (5) ±6
2 and 5	Std. Diet + 10% CO_2	657 (13) ±119	365 (5) ±40
3	Std. Diet + 15% CO_2	535 (5) ±79	378 (4) ±4
6	Std. Diet + 10 and 15% CO_2	781 (4) ±113	
7	E.D. Diet only	537 (4) ±77	313 (5) ±4
8	E.D. Diet + 10% CO_2	811 (9) ±176	314 (5) ±3

* μMole NH_3 per 100 mg. dry kidney tissue per hour.
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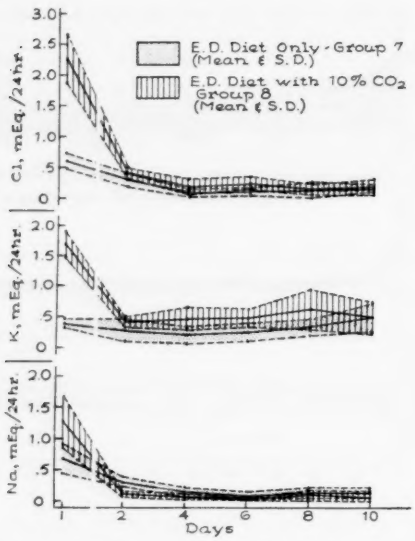

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cent CO_2 alone. Urine pH was lower (mean, 5.8) than that found in respiratory acidosis alone (mean, 6.2 to 6.8). Renal glutaminase activity was 2,228 ± 371 mMole per 100 mg. dry tissue per hour as contrasted to a mean value of 657 for respiratory acidosis alone.

DISCUSSION

The pattern of urinary acid excretion during respiratory acidosis differs markedly from that resulting from other forms of acid loading. In the present study, there was initially a small but significant rise in the excretion of acid (both as ammonia and titratable acid) during the first day or two of acidosis; thereafter, despite continued, severe, systemic acidosis, urinary acid excretion returned virtually to control values. By contrast, the administration of an acid buffer, such as sodium acid phosphate, promptly increases the excretion of titratable acid as long as the acid load is given, even though the amounts of acid cause only a slight lowering of the concentration of serum bicarbonate (24).

The character of acid excretion during ammonium chloride acidosis differs even more strikingly from that encountered during respiratory acidosis. The administration to rats of small amounts of a strong acid, *e.g.*, 2 mMole NH_4Cl daily, results in the following response (13): *1*) Acid excretion (almost entirely as ammonia) rises over a five day period to a maximum level. *2*) This level is maintained throughout the duration of the acid loading. *3*) Despite the absence of changes in the pH or concentrations of bicarbonate in blood (or at most, minor depressions), far more acid is excreted than in control animals. *4*) Indeed, acid excretion is directly proportional to the magnitude of the strong acid load (whereas in respiratory acidosis, 10 and 15 per cent CO_2 elicit the same minor rise in urinary acid). The sharp dichotomy between the profound degree of systemic acidosis (blood pH 7.15 ± 0.07 in 15 per cent CO_2 group) on the one hand and the comparatively minor rise in acid excretion on the other constitutes one of the most striking features of the pattern of acid excretion during respiratory acidosis. The failure of respiratory acidosis to produce a sustained increase in renal acid excretion cannot be attributed to the fact that as plasma pCO_2 increases, carbonic acid can be excreted via the lungs and therefore would not require renal compensation beyond the initial period, since the blood pH was markedly acid throughout the period of study.

Unlike metabolic acidosis, the serum bicarbonate rises during CO_2-rebreathing. This rise begins within the first hour and reaches a maximum value in about one day, after which the elevated serum concentration is maintained throughout the duration of exposure to CO_2 (25). It is probable that the absence of greater augmentation of acid excretion is linked with this elevation of serum bicarbonate. During the early period, before the concentration of serum bicarbonate has reached maximum values, mean urine pH falls below control levels; the excretion of ammonia and titratable acid is therefore accelerated. After the first day, the urine becomes more alkaline. Although the elevated pCO_2 of blood accelerates bicarbonate reabsorption, it is probable that a portion of the greatly increased load of filtered bicarbonate escapes into the urine, especially when the serum bicarbonate concentration is maximal, rendering it more alkaline. In turn, the more alkaline urine may serve to suppress the excretion of ammonia and titratable acid.

The early rise in the concentration of serum bicarbonate is roughly paralleled by a commensurate fall in serum chloride. This fall in filtered chloride and rise in filtered bicarbonate could result in a sharp reduction in the proportion of chloride as compared with bicarbonate reaching the exchange site in the distal tubule. It is especially noteworthy, in this connection, that the administration of 3 mMole NH_4Cl to rats during respiratory acidosis markedly accelerated NH_3 excretion and lowered urine pH, at the same time that renal glutaminase was activated to levels (2,228 ± 371 mMole NH_3 per 100 mg. dry tissue per hour) not significantly different from that found in rats subjected to 3 mMole NH_4Cl daily alone (Figure 5). It is likely that the NH_4Cl load, by reducing the concentration of bicarbonate and raising the concentration of chloride in serum, diminished the amount of bicarbonate reaching the distal tubule, at the same time that the delivery of NaCl to the exchange site was increased. As a result, ammonia excretion was accelerated

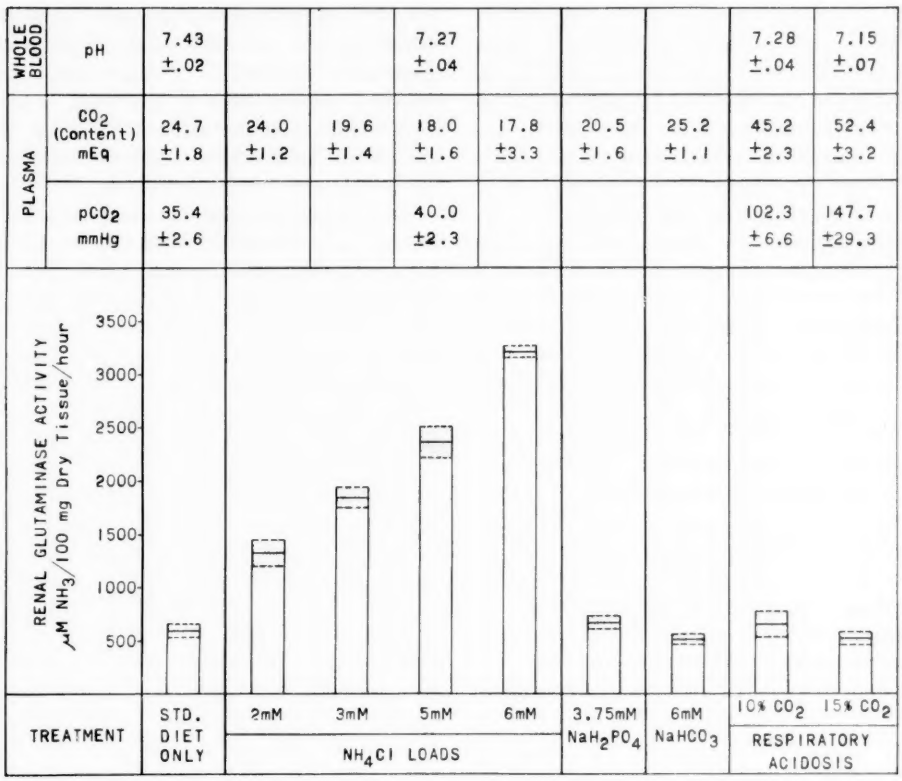

FIG. 5. RESPONSE OF RAT RENAL GLUTAMINASE ACTIVITY TO VARIOUS EXPERIMENTAL CONDITIONS

and this, in turn, could have activated renal glutaminase.

In keeping with the minor and transient rise in ammonia excretion during respiratory acidosis is the finding that renal glutaminase is not activated. Figure 5 summarizes the response of this enzyme system to various acid-base loads, as found in our laboratory. Glutaminase adaptation is observed only when ammonia excretion is persistently increased, a circumstance that prevails during chronic administration of a strong acid (NH_4Cl) load. Since CO_2-rebreathing results in a far more severe systemic acidosis (and intracellular acidosis as well), it seems likely that activation of the glutaminase enzyme system cannot be ascribed either to extracellular or intracellular acidosis alone. It is possible that the activation of glutaminase is attributable to product removal (*i.e.*, ammonia excretion); any circumstance tending to accelerate the excretion of ammonia should then result in an activation of the ammonia-producing enzyme systems of the rat.

Although acid excretion is elevated to only a minor extent in respiratory acidosis, the reabsorption of large amounts of filtered bicarbonate indicates that hydrogen ion secretion is increased (*26, 27*). This augmentation in bicarbonate reabsorption and hydrogen ion secretion is best correlated with the rise in tension of CO_2 in plasma (*5, 7, 28, 29*). In chronic respiratory acidosis in the dog, there is a further increase in bicarbonate reabsorption above that which might be predicted for any particular plasma pCO_2 value (*8*). That this apparent adaptation to prolonged respiratory acidosis might be mediated by means of the specific adaptation of renal carbonic anhydrase would seem a distinct possibility. Killion and Schaefer have reported increased renal carbonic anhydrase activity occurring within one hour in the kidneys of rats and guinea pigs exposed to an atmosphere

containing 30 per cent carbon dioxide (30). However, in our more chronic experiments, in atmospheres with less carbon dioxide content, we were unable to show increased activity of this enzyme. The explanation of the progressively more efficient bicarbonate reabsorption at any given pCO_2 during chronic respiratory acidosis is therefore not forthcoming from these studies.

In vitro studies with whole blood have shown that when exposed to carbon dioxide, chloride in the plasma enters the erythrocyte (31, 32). In acute respiratory acidosis in man (2, 3) and the nephrectomized dog (3), this mechanism has been shown to be important in producing hypochloremia. However, that an added mechanism for hypochloremia is the urinary loss of chloride had not been clearly demonstrated until the studies of Epstein, Branscome and Levitin (11). In our experiments, an early, marked, negative balance of chloride was likewise observed. Although the shift of chloride from the extracellular space into the erythrocyte does promote hypochloremia, it is apparent that the early loss of chloride in urine is also an important factor.

The excretion of potassium is slightly increased throughout the period of study. In acute respiratory acidosis in man of one hour duration, potassium excretion is depressed (5), presumably because increased H^+, generated from CO_2 in the tubular cell, rather than K^+, exchanges for reabsorbed Na^+. The persistently elevated potassium excretion, despite lowered serum concentrations, may be the consequence of accelerated delivery of $NaHCO_3$ to the distal tubule as serum bicarbonate rises, a circumstance which could increase the Na^+-K^+ exchange and thus account for the potassium loss. If increased amounts of $NaHCO_3$ are delivered to the distal tubule, the exchange of Na^+ for H^+, as well as for K^+, is in all likelihood augmented, but the enhanced secretion of H^+ into bicarbonate-containing tubular fluid would not augment urinary acid (NH_3 plus titratable acid) excretion.

The finding of a lowered plasma concentration of potassium in the experimental groups of this study contrasts with the elevated concentrations found in acute respiratory acidosis (2, 3, 33), where potassium has been shown to enter the extracellular space, presumably from cells. Similarly, the relationship of potassium concentration to blood pH observed in human subjects with disturbances in acid-base balance (34) was not apparent in this study. It seems likely that respiratory acidosis does in fact cause a transfer of potassium into the extracellular space, but in our chronic studies renal losses probably prevent the usually observed hyperkalemia.

Our finding of a slightly decreased muscle potassium is in agreement with the observation of Levitin, Jockers and Epstein (35). The previously noted potassium shift into the extracelluar space, together with the observed loss of potassium in the urine, is in keeping with the finding of lowered muscle potassium. The lowering of both muscle potassium and sodium in respiratory acidosis could be a consequence of exchange of extracellular hydrogen ion for intracellular anions. Decreased muscle sodium in metabolic acidosis has been previously reported, whereas with an adequate intake of potassium, muscle potassium is usually not lowered by metabolic acidosis (13, 36, 37). Thus, in both metabolic and respiratory acidoses, the sodium of muscle is decreased, whereas only respiratory acidosis significantly lowers muscle potassium. The apparent restriction of pH change principally to the extracellular fluid in metabolic acidosis (38, 39) may in some way explain the difference in response of muscle potassium in metabolic and respiratory acidoses. As suggested by Fenn, Rogers and Ohr, the lowering of muscle sodium in both metabolic and respiratory acidoses may not represent a simple exchange of intracellular sodium for extracellular hydrogen ion, but rather an increased ability of the sodium pump to deliver sodium into a more acid medium (40).

Bone, as an extracellular repository for sodium and potassium, has been recognized as a potential source from which the urinary excretions of these anions during acidosis can be derived (22). That in metabolic acidosis bone sodium is released, apparently in exchange for hydrogen ion, has been demonstrated (22, 37, 41). Bergstrom and Wallace (22) observed a similar role for bone potassium, but Levitt, Turner, Sweet and Pandiri (37) failed to find changes in bone potassium in rats with metabolic acidosis. In respiratory acidosis, no change was found by us in bone sodium, confirming a previous observation (35). It is possible that the chronicity of our experiments, as

compared with the more acute studies of metabolic acidosis, would explain the differences observed in bone sodium. Bone potassium was slightly elevated in respiratory acidosis. Thus, in chronic respiratory acidosis, participation of bone sodium and potassium in the buffering of carbonic acid is not apparent.

SUMMARY

The pattern of renal and cellular responses of electrolytes to chronic respiratory acidosis was studied in rats.

Urinary acid excretion (NH_3 plus titratable acid) increased transiently for the first day or two and then returned to control values despite continued severe respiratory acidosis. A slight rise in urine pH, presumably due to increased bicarbonate excretion as serum bicarbonate reached maximal values, appeared to be chiefly responsible for the rapid return of urinary ammonia to control values. Renal glutaminase was not activated during respiratory acidosis; this is strong evidence against the role of intracellular or extracellular pH as regulatory factors in the adaptation of renal ammonia-producing enzymes. In spite of increased tubular bicarbonate reabsorption, as evidenced by the stabilization of serum bicarbonate at high levels, adaptation of renal carbonic anhydrase did not occur.

Chloride excretion was greatly elevated the first day of respiratory acidosis but thereafter returned to control values. Potassium excretion was also markedly elevated the first day and continued to be excreted at a slightly increased rate for the remainder of the study.

Muscle sodium and potassium were slightly decreased after 11 days of respiratory acidosis. The most striking change in bone electrolytes was a fall in magnesium content.

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THE ROLE OF THE MONKEY FETUS IN THE EXCHANGE OF THE WATER AND SODIUM OF THE AMNIOTIC FLUID *

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Isotopic tracers allow for the expedient investigation of the exchange of elements or metabolic products between the maternal organism and conceptus. The quantity of carrier transferred per unit of time can be estimated from the behavior of tracers if the experimental data are subjected to the proper mathematical treatment. The general background and practical applications of the theory of multi-compartment systems to related biologic problems have recently been reviewed (1). Depending upon the aim of the investigation and the nature of the available experimental data, one can subdivide a given system into an arbitrary number of hypothetical compartments which exchange, or are assumed to exchange, their constituents at constant rates. If such a system is to be completely defined, time-activity curves for all compartments must be known and all must be accessible. Once the applicability of a certain kinetic model is established, the transfer rates can be calculated from a minimum of experimental data. It is the purpose of these investigations to test the applicability of a proposed kinetic model in order to provide the theoretical basis for further studies on clinical material.

It is known that the human fetus participates in the exchange of water and electrolytes between amniotic fluid and mother (2), but because of its inaccessibility a quantitative formulation of these mechanisms is not easily accomplished. A knowledge of the tracer concentration in fetal blood as a function of time is a prerequisite for the calculation of transfer rates. Such a time-activity curve for the fetal compartment can be determined directly on some experimental animals where samples of fetal blood can be obtained without disturbing the continuity of the system. The pregnant primate, a miniature model of the human maternal organism, should serve this purpose very well if it can be shown that the two species are comparable in the more pertinent aspects of water and electrolyte exchange.

For the study of these problems three sets of experiments were carried out. The first was intended to show that the transfer rates of water in and out of the amniotic fluid compartment of pregnant monkeys at term are identical within the limits of error of the method. The second was intended to estimate the ratio of water to sodium transfer and to compare this ratio with values previously reported for the human. Finally, attempts were made to determine time-activity curves for all three compartments simultaneously and to estimate the overall transfer rates involving the fetus *in utero*.

METHODS

a) Analytic procedures. The deuterium oxide in these body fluids was determined by the falling drop method (3). For the determination of tritium, the samples were combusted and the resulting water converted into hydrogen gas by the method of Graff and Rittenberg (4). A known amount of hydrogen was then transferred to Ballentine tubes and filled to atmospheric pressure with methane as quench gas (5). Disintegrations were measured in the proportional region and all results expressed uniformly as counts per minute (cpm) per 50 mm. of mercury of hydrogen gas. The activity of samples containing sodium-22 was estimated with a well-type scintillation counter. The concentration of sodium ion was determined by the flame photometric technique.

b) Physiologic preparation. The operative procedures varied slightly according to the experiment. Pregnant rhesus or cynomolgus monkeys at term, as shown by X-ray or known ages of gestation, were anesthetized

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with intravenous Nembutal® in doses of about 30 mg. per Kg. A small incision was made over Poupart's ligament, the femoral vein isolated, and a polyethylene catheter introduced in a distal direction. The proximal portion of the femoral vein was tied with silk. The uterus was delivered through a midline incision and, after identification of the placental margins, a purse-string suture was placed in the lower uterine segment or near the dome of the fundus. After puncture with a number 15 gauge needle through the area of myometrium surrounded by the purse-string suture, a multi-holed polyethylene catheter (Clay-Adams, size PE 90) was introduced into the amniotic cavity. The needle was withdrawn over the catheter and the purse-string suture tied.

In those experiments where fetal blood samples were desired, interplacental vessels were isolated as described by Reynolds, Paul and Huggett (6). After isolation of the vessels the distal portion leading to the secondary placenta was tied, the artery or vein punctured with a thin-walled 18 gauge needle containing a Clay-Adams PE 50 polyethylene catheter, and the catheter secured with two silk ties. The myometrium was approximated with a series of interrupted silk sutures. Intravascular clotting of fetal and maternal blood was prevented by the introduction of small amounts of heparin.

The total body water and total exchangeable sodium were either determined by routine procedures several days before the experiment or estimated by extrapolation of the tracer curves. The fetal body water content was estimated at 65 per cent of body weight and the volume of the amniotic fluid was determined by the Congo Red dilution method and checked by extrapolation of tracer concentration to zero time as described by Neslen, Hutchinson, Hallet and Plentl (7). Mixing of dye and tracer solutions with the amniotic fluid was accomplished mechanically by frequent withdrawal and reinjection of the fluid.

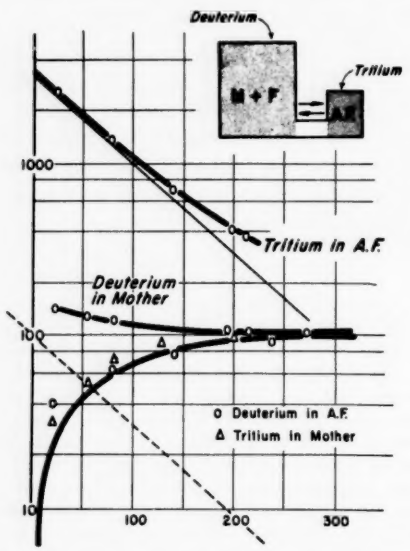


FIG. 1. SPECIFIC ACTIVITY OF TRITIUM AND CONCENTRATION OF DEUTERIUM AS A FUNCTION OF TIME IN MINUTES

As shown on the kinetic model (insert) deuterium was injected into the maternal circulation and tritium introduced into the amniotic fluid. The simultaneous transfer in opposite directions can be calculated from the disappearance and appearance constants for the two tracers.

Tracers were introduced into one or more of the three compartments as described for each of the following experiments. Samples of maternal venous blood, fetal blood and amniotic fluid were obtained at intervals of five to 30 minutes over a period of three to five hours as indicated in each case. The blood samples were collected in tubes containing crystalline heparin, centrifuged and the supernatant plasma used for isotope determinations. Amniotic fluid samples were collected without special precautions and were stored in the frozen state. After a predetermined interval the experiments were terminated and the fetus delivered by cesarean section.

THEORY

Since only tracers for water and sodium were used, distribution phenomena rather than metabolic processes are considered in this study. For the mathematical treatment of the data the simplest hypothetical model is used which is not in conflict with known possibilities and representations of the system. The analysis of the data then makes it possible to calculate either the transfer rates or the size of the compartments. In the following section the theoretic background for the two- and three-compartment case is briefly discussed and a specific example is given for each.

a) *The two-compartment case.* First, a broad division into maternal organism and products of gestation can be made. The numerous transfers within each of them, *e.g.*, exchanges within the fluid compartments of the maternal or fetal organism, would not appreciably influence the overall exchange mechanisms between mother, fetus and amniotic fluid provided these major exchange rates are appreciably slower. Under these circumstances the slowest, measurable rate constitutes the rate-determining step.

Wide differences in the major transfer rates may lead to further simplifications of such systems. Since there is reason to believe that the exchange rates between mother and fetus are rapid by comparison to the exchange rates between the amniotic fluid and either of the other compartments, mother and fetus may be "lumped" into one. The simplest possible system is then composed of two, rather than three compartments, exchanging their species according to the scheme:

$$\text{Amniotic fluid} \rightleftarrows \text{fetal} + \text{maternal systems.}$$

There is no detectable change in the size of either compartment during the experimental period; hence, the rates in both directions must be equal.

The calculation of the exchange rate necessitates a knowledge of: *1*) the size of the combined maternal and fetal compartments, *2*) the size of the amniotic fluid compartment, and *3*) the disappearance constant. The experimental procedures for *1*) and *2*) have been described and the disappearance constant is determined graphically as follows.

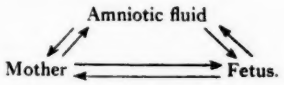
After injection of the tracer into either major compartment, serial samples are taken at arbitrarily chosen intervals. The experimental points for amniotic fluid and maternal plasma which approach an equilibrium value fall on exponential curves. From a knowledge of the quantity of tracer injected, the theoretic "equilibrium" value can be calculated. Thus for the experiment illustrated graphically in Figure 1, the total body water was 3,700 ml. and the amount of deuterium oxide injected, 11.0 Gm. The theoretic equilibrium value can then be calculated as 0.297 per cent deuterium oxide by weight. Subtracting this constant from each of the experimental points gives a straight line on a semilog plot against time, the slope of which is -0.756 per hour. The volume of the amniotic fluid had been determined as 110 ml.; hence the combined maternal and fetal volume is 3,590 ml. Substituting these values in Equation *1*

$$\rho = -S_1 \frac{rS_2}{S_1 + S_2} = 3{,}590 \frac{(0.756)(110)}{3{,}700}$$

$$= 80.7 \text{ ml. per hour} \quad 1$$

a derivation of which is given in the Appendix, gives a value of 80.7 ml. or 4.5 moles per hour for the exchange rate between the two compartments.

b) *The three-compartment case.* The quantitative formulation of the exchanges involving the fetal system requires a more complicated theoretic system. A minimum of three compartments must be assumed, all of which could exchange with each other. Such a three-compartment system can be represented schematically.



During the brief period of the experiment there is no change in size of any compartment; hence the sum of the transfer rates *in* and *out* of each compartment must be equal, *i.e.*, the steady state conditions are met. Each of the six transfer rates may be different but the sum of these rates for each compartment must be zero. A positive value is assumed for each individual transfer rate. During the experiment no new tracer or nonisotopic material enters the system and no appreciable amount is lost, *i.e.*, the system is "closed." Although the concentration and amount of tracer in each compartment changes with time, the total amount of tracer in all three compartments at any time is constant. At least in theory, therefore, a knowledge of two of the disappearance curves should suffice for the calculation of the third disappearance or appearance curve. The maternal compartment is more than 10 times larger than the fetal or amniotic fluid compartment and this extreme difference introduces an enormous error for a time-activity curve derived in this manner. Independent measurements must, therefore, be made for each compartment.

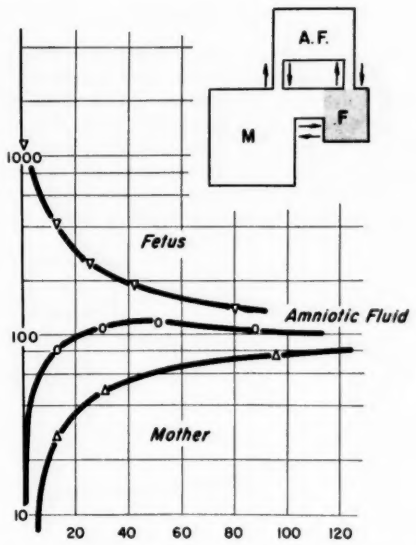


FIG. 2. SEMILOG PLOT OF THE SPECIFIC ACTIVITY FOR TRITIUM IN THE THREE COMPARTMENTS AS A FUNCTION OF TIME IN MINUTES

The insert shows the kinetic model, the shaded area representing the primary compartment (fetus).

The curves for each of the three compartments are described by the general equations:

Tracer concentration in:

$$\text{Amniotic fluid} = \alpha_1 e^{r_1 t} + \alpha_2 e^{r_2 t} + \text{equilibrium}$$

$$\text{Mother} = \beta_1 e^{r_1 t} + \beta_2 e^{r_2 t} + \text{equilibrium}$$

$$\text{Fetus} = \gamma_1 e^{r_1 t} + \gamma_2 e^{r_2 t} + \text{equilibrium.}$$

A semilog plot of the experimental data can be used to calculate the exponential constants r_1 and r_2 and the integration constants are obtained by extrapolation to zero time. As a specific illustration, the curves reproduced in Figure 2 can be expressed:

$$\text{Amniotic fluid} = 3740e^{-0.955t} + 75e^{-7.72t} + 100$$

$$\text{Mother} = -110e^{-0.955t} + 10e^{-7.72t} + 100$$

$$\text{Fetus} = 170e^{-0.955t} - 270e^{-7.72t} + 100$$

which defines the integration and exponential constants. The maternal body water in this particular experiment was determined as 3,470 ml., the amniotic fluid as 95 ml. and the fetal body water was, retroactively, estimated as 155 ml. By substituting these values in Equations *8*, *9*, *6* and *10* of the Appendix, the six individual transfer rates can be calculated.

EXPERIMENTAL

A total of 19 rhesus and cynomolgus monkeys was used. Only those experiments were considered valid in which a live fetus was obtained. Some of the failures were due to inherent complications rather than faulty technique. As shown in Table I about a quarter of all the animals had only a single placenta, and in these access to the fetus without entry into the amniotic cavity was not possible. In animals where a double placenta was encountered, the primary placenta was located on the anterior wall two-thirds of the time. Although not essential, it is nevertheless desirable to cannulate the vessels in the direction of the primary placenta since this is more likely to lead to a representative sample of fetal blood.

TABLE I

The incidence and location of single and double placentae in 17 pregnant rhesus and two pregnant cynomolgus monkeys

	Number	Per cent
Single placenta	5	26.3
Anterior	1	5.3
Posterior	4	21.0
Double placenta	14	73.7
Primary anterior	10	52.6
Primary posterior	4	21.0

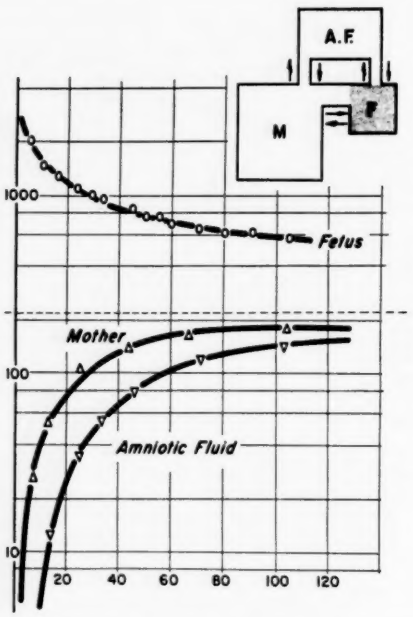


FIG. 3. SEMILOG PLOT OF RELATIVE RADIOACTIVITY IN FETUS, MOTHER AND AMNIOTIC FLUID AS A FUNCTION OF TIME IN MINUTES FOLLOWING THE INJECTION OF SODIUM-22 INTO THE FETAL CIRCULATION

The dotted horizontal line is the calculated equilibrium value based on a knowledge of the total exchangeable sodium. The insert shows the kinetic model.

1) Transfers to and from the amniotic fluid. After a series of preliminary experiments to evaluate the surgical and physiological techniques, the water exchange rate of the amniotic fluid was determined using tritium and deuterium simultaneously. In the first experiment tritiated water was injected into the amniotic sac and deuterium oxide into the maternal circulation. The results of tracer analyses of amniotic fluid and maternal plasma on serial samples, both expressed on a comparable scale, are shown graphically in Figure 1.

In five subsequent experiments sodium-22 and tritium were injected simultaneously into the amniotic sac and the transfer rates calculated in the manner described above. The significant data for each of these experiments are given in Table II.

2) Transfers involving the fetus. a) Primary compartment: Fetus. Three experiments were carried out in which the tracers were introduced into the fetal compartment by injection into the interplacental vessels. One representative experiment where tritium was employed is reproduced in Figure 2. Despite the apparently adequate number of experimental points, the error for exponential and integration constants was found to be too great for an accurate estimation of all six transfer rates. Qualitatively, the pattern was consistent in all

TABLE II

Summary of significant data on experiments designed to determine water and sodium transfer from amniotic fluid to maternal and fetal organisms

	Monkey No. 160	Monkey No. 262	Monkey No. 264	Monkey M.T.	Monkey No. 54
Total body water (*ml.*)	4,780.0	4,500.0	3,600.0	2,250.0	3,100.0
Amniotic fluid (*ml.*)	116	95	105	95	120
Total sodium in A.F. (*moles*)	0.012	0.014	0.010	0.0099	0.0132
Disappearance constant for water ($N/hr.$)	0.834	0.945	0.647	1.26	3.18
Disappearance constant for sodium ($N/hr.$)	0.192	0.157	0.160	0.346	0.840
Water transfer (*moles/hr.*)	5.4	5.0	3.8	6.65	21.2
Sodium transfer (*moles/hr.*)	0.0023	0.0022	0.0016	0.0036	0.0138
Ratio of H_2O:Na transfer $\times 10^3$	2.3	2.3	2.0	1.8	1.5

TABLE III

Significant data on experiments designed to determine retention curves for sodium-22 of fetal blood, defined by the expression:

$$C_1e^{-r_1t} + C_2e^{-r_2t} + C_3$$

	Monkey No. 32-64	Monkey No. 32-66	Monkey No. 32-68
Sodium content of fetus (*mEq.*)	9.68	14.9	22.3
Retention curve for sodium:			
C_1	0.1690	0.318	0.440
C_2	0.7887	0.629	0.480
C_3	0.0423	0.053	0.080
r_1 (per hour)	0.346	0.84	0.714
r_2 (per hour)	10.39	8.28	6.93
Total transfer of sodium (*mEq./hr.*)	78.7	81.4	74.3

three experiments. Under these conditions, the assumed closed three-compartment system leads to a time-activity curve for the amniotic fluid which passes through a maximum and eventually approaches the equilibrium value from above.

When radiosodium was injected into the fetus as the primary compartment a series of curves similar to that shown in Figure 3 was obtained. Three additional experiments were carried out in which sodium-22 and tritium were administered simultaneously into the fetal circulation and none of them yielded experimental data suitable for the calculation of all six transfer rates. Satisfactory retention curves for the fetal blood compartment were used for the calculation of combined extra- and intrafetal sodium exchange. The analysis of these retention curves is given in Table III.

b) Primary compartment: Amniotic fluid. When the amniotic fluid is chosen as the primary compartment and tritium is injected into it, the resulting disappearance curves can be used for a very accurate mathematical analysis. The experimental data on one such run are given graphically in Figure 4 and the final results on two additional experiments are presented in Table IV. The numerous operations in the graphic analysis and the calculation of each transfer rate lead to an accumulation of errors which is taken into account in the tabular presentation of the results. Although the measurements are relatively crude, the final limits of error for the majority of the transfer rates were found to be surprisingly narrow.

DISCUSSION

Based on a simplified two-compartment system an accurate estimation of the transfer rates of water and sodium *from* the amniotic fluid can be made. The amniotic fluid is the only compartment in this system in which "instantaneous" mixing of the tracer is possible. Except for purely mechanical complications such as an insufficient quantity

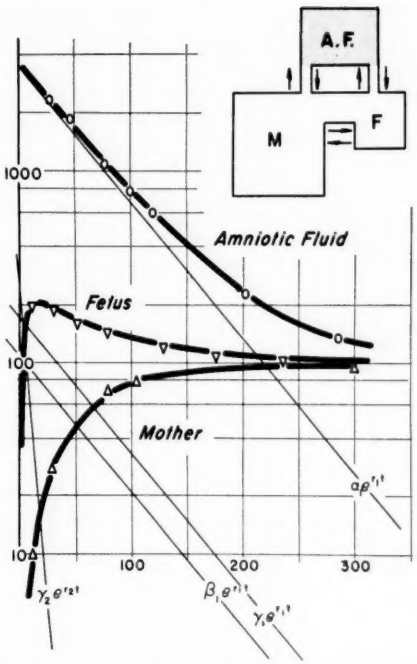


FIG. 4. SEMILOG PLOT OF TRITIUM CONCENTRATION VERSUS ELAPSED TIME IN MINUTES

The specific activity for all compartments is referred to a calculated equilibrium value arbitrarily set at 100 per cent. Insert shows the kinetic model; the shaded area the primary compartment (amniotic fluid). The straight lines represent the exponential terms from which the rate constants are calculated.

TABLE IV

*Transfer rates for water as calculated from time-activity curves of maternal, fetal and amniotic fluid compartments**

	Monkey No. 266	Monkey No. 258
Fetus to mother	910 (890–930)	1,103 (993–1,212)
Mother to fetus	934 (922–946)	1,074 (1,018–1,130)
Fetus to amniotic fluid	62 (43–80)	47 (0–94)
Amniotic fluid to fetus	57 (51–63)	76 (69–83)
Amniotic fluid to mother	11 (5–17)	12 (6–25)
Mother to amniotic fluid	12.5 (0–25)	43 (0–87)

* Tritiated water was injected into the amniotic fluid which served as the primary compartment. The results are tabulated in ml. per hour; the values in parentheses represent limits of error compatible with measured experimental data.

of amniotic fluid which might lead to pocket formation and poor sampling, the retention curve for this compartment is reliable. This is clearly illustrated in the first experiment (Figure 1) wherein two tracers were introduced into maternal organism and amniotic fluid, respectively, in order to measure the transfer rate in opposite directions simultaneously on the same animal. The retention curve for the amniotic fluid follows an exponential pattern almost exactly, while the maternal curves are not well suited for analysis. A distribution of the tracers into maternal subcompartments (one of which is the fetus) takes place at rates which cannot be neglected in the mathematical treatment. The maternal curve cannot be represented by a single, perhaps not even by a double exponential curve, but in the two-compartment case these deviations are of relatively little importance if only the total transfer rate, *i.e.*, the amount leaving (and entering) the amniotic fluid, is to be determined. When the disappearance of sodium was measured, simultaneously the retention curve for the amniotic fluid compartment was found to be governed by one exponent only. The slow distribution and equilibration of sodium in the maternal compartment makes it difficult to calculate the transfer rates in the reverse direction, *i.e.*, from the combined maternal and fetal compartments to the amniotic fluid.

In Table V the mean values obtained for pregnant monkeys are compared with similar values previously reported for the human (8, 9). On a weight basis, the water exchange of the amniotic fluid in pregnant monkeys is about three times faster than that of humans. The exchange of sodium in the two species bears a similar relation; hence the ratio of sodium to water transfer is nearly identical. At least in this respect the two species are comparable.

There exists indirect evidence that part of the water exchange of the amniotic fluid takes place through the intermedium of the fetus. Paul, Enns, Reynolds and Chinard (10) determined the exchange of the amniotic fluid in pregnant rabbits where the fetus had been crushed *in utero* and compared results with those obtained in a control series. The transfer rate in the former group was about one-half that of the latter, a fact interpreted by the authors as evidence that, in the rabbit, about one-half of the transfer takes place via the fetus. The fetal membranes apparently are of less significance since tying the vitelline vessels did not affect the transfer rates. In the human, the fetus also plays an important part in this exchange (2). Within a few minutes after the injection of labeled water into the amniotic fluid of pregnant women at term, the tracer concentration in cord blood exceeds the calculated equilibrium value by a factor of two or three. If the time interval from injection of tracer to sampling of fetal blood exceeds a period of three hours, the tracer concentration in the fetus is about equal to or only slightly higher than the calculated equilibrium value. Although the exact shape of the fetal tracer concentration curve is not known, it must, at some time, pass through a maximum. It could be shown that this is only possible if more than 25 per cent of the transfer of water from amniotic fluid to mother takes place through the fetus. In the present series of experiments on monkeys the shape of the fetal curve could be determined directly and, under specific conditions, an accurate quantitative determination of the transfer rates could be made.

TABLE V

Comparison of the average transfer rates for water and sodium from amniotic fluid to maternal organism in humans and monkeys at term

	Human	Monkey
Amniotic fluid volume (*ml.*)	785	107
Water transfer (*moles/hr.*)	26.6	8.4
Sodium transfer (*moles/hr.*)	0.0125	0.0047
Ratio of H_2O:Na transfer $\times 10^3$	2.1	1.8

If it is assumed that the three compartments, amniotic fluid, mother and fetus, exchange water with each other, and if all three compartments are accessible, any one of them may serve as the primary compartment. The large size and poor initial mixing of the maternal pool make it unlikely that it would be suitable as a primary compartment if the experimental data are collected for the purpose of calculating transfer rates. On purely theoretical grounds, therefore, no attempts were made to use this approach.

Injection of tritiated water into the fetal compartment (Figure 2) gives a retention curve which falls off rapidly and soon approaches the equilibrium value. The time-activity curves for amniotic fluid and maternal compartments rise

unequally and also approach the equilibrium value, the former from above and the latter from below. Because of the relatively large analytic error inherent in these data neither curve can be used for the accurate determination of the exponential constants. The fetal curve, on the other hand, should serve this purpose reasonably well. The initial rapid disappearance of the tracer from the fetal compartment is, to some extent, a mixing phenomenon but due mostly to the fast exchange between mother and fetus. The fetal compartment, like the maternal compartment, consists of a number of sub-compartments; these may be expected to distort the time-activity curves. Furthermore, the small difference between the experimental points and the equilibrium value near the tail end of the fetal curve renders the first exponential constant inaccurate and this, in turn, leads to a cumulative error for the second exponential constant which governs the initial sharp decline of the curve. Neither constant is reliable and the experimental data obtained by using the fetus as the primary compartment are therefore of qualitative significance only. They cannot be used for the calculation of the transfer rates within this system.

If the tracer is introduced into the amniotic fluid compartment, a mathematical analysis of the data is possible. Almost instantaneous mixing can be accomplished by physical means, virtually impossible in the two preceding cases. This results in a reliable retention curve for at least one of the three compartments; hence, the first exponential constant can be estimated with a very small margin of error. Since this constant is fixed within narrow limits, the errors for all the values derived from it are correspondingly smaller and the two integration constants for the fetal compartment can be determined with considerable accuracy. These, in turn, determine the corresponding values for the maternal compartment. An accurate knowledge of the maternal tracer concentration is, therefore, not necessary and the calculation of the transfer rates is not dependent upon such data. Moreover, the concentration of the fetal compartment throughout the most significant portion of the experiment is within the optimal range for all analytic methods. Under these conditions, the delayed mixing in the fetal compartment does not appreciably affect the values of the final transfer rates. In Table IV the six calculated transfer rates and the greatest possible errors compatible with the experimental data are given for two such experiments. The two animals were of nearly identical weight and differed only slightly in the weight of their fetuses and the volume of the amniotic fluid. The transfer rates for both animals are comparable within the experimental error of the method. Between fetus and mother there exists a molecular exchange equivalent to about 1 L. of water per hour. On a weight basis this would correspond to about 4,200 ml. of water per hour per Kg. of fetus, more than four times the value estimated for the human fetus at term. The total exchange of the water of the amniotic fluid, that is, the sum of its exchange with fetus and mother, corresponds to the rough estimates given above. As predicted, more than three-quarters of this exchange takes place through the fetus. Thus for monkey No. 266 the total amount of water leaving the amniotic fluid compartment could be a maximum of 68 ml. per hour and of this a minimum of 5 (7.3 per cent) and a maximum of 17 ml. (25 per cent) would pass directly to the mother. The limits of error for the transfer rates in the reverse direction, *i.e.*, from fetus to amniotic fluid and mother to amniotic fluid, are somewhat broader. The direct exchange between mother and amniotic fluid, though small compared to the exchange between amniotic fluid and fetus, is nevertheless real and essential for the continuity of this system.

Numerous attempts to analyze the data of the experiments where sodium-22 was used failed to yield ranges for transfer rates suitable for interpretation. The reasons for this have been outlined above and these factors become even more significant in the mathematical treatment of the three-compartment case. The addition of several slower compartments in mother and fetus leads to a complex mathematical analysis, which is not justified on the basis of the experimental data. A direct exchange between amniotic fluid and mother is not established but probably exists. The experimental curves only indicated that there is a rapid exchange of sodium between mother and fetus and a slower one between amniotic fluid and fetus. The ratio of these exchange rates is proportional to the sodium content of the two compartments.

If the average disappearance rate of sodium from the amniotic fluid is taken as about 4.7 mEq. per hour (Table II), the quantity exchanged between fetus and mother is probably between 60 and 70 mEq. per hour. This estimated range for extra-fetal exchange must be lower than the values calculated for the total loss of sodium from the fetal blood compartment. Injection of radiosodium into the fetal blood stream yields retention curves which can be used to calculate this loss. The retention curve is given by the expression:

$$z = C_1e^{-r_1t} + C_2e^{-r_2t} + C_3 \qquad 2$$

where z is the specific activity for sodium or tritium, C_1, C_2 and C_3 integration constants expressed as a fraction of one. The quantity of carrier leaving this compartment per unit of time is related to this expression by:

$$\frac{-K}{S_z}z = \frac{dz}{dt} \qquad 3$$

where K represents the sum of all transfers *out*, and S_z the size of the compartment, *i.e.*, its sodium or water content. Since this equation holds for all values of z, at $t = 0$, $z = 1$ and the total amount leaving the fetus:

$$-K = Sz(-r_1C_1 - r_2C_2). \qquad 4$$

In Table III the values obtained in this manner are compared for three animals of approximately equal weight. A total of 80 mEq. of sodium was found to leave the fetal blood stream per hour.

SUMMARY

The water and sodium exchange mechanism between mother, fetus and amniotic fluid was investigated in a series of pregnant monkeys at term. A number of likely kinetic models was assumed and their mathematical analyses reviewed. The total exchange rates for water and sodium of the amniotic fluid were determined under the assumption of closed two-compartment systems. An average of 8.4 moles of water and 4.7 mEq. of sodium were found to exchange per hour. Although the quantity exchanged is three times greater than that found for the human, the ratio of transfer rates of water and sodium is identical for the two species.

The role of the fetus was then investigated under the assumption of a closed three-compartment system, represented by amniotic fluid, fetus and mother. The data obtained in experiments where the fetus was used as the primary compartment were not sufficiently accurate for the desired calculations. Time-activity curves for the experiments where the amniotic fluid was the primary compartment were suitable for curve analysis and the six theoretic transfer rates could be calculated with a known margin of error. Between mother and fetus there exists a molecular exchange equivalent to 1,000 ml. of water per hour; between fetus and amniotic fluid about 50 ml. per hour; and the direct exchange between amniotic fluid and mother has a range of 5 to 20 ml. per hour. The primate fetus transmits at least 75 per cent of the water leaving the amniotic fluid.

For labeled water the closed three-compartment system represents the pregnant organism reasonably well and a mathematical analysis can be applied. Tracers for sodium, on the other hand, when used in identical manner led to superficially similar conclusions but the simple closed three-compartment system does not reflect the exchange mechanisms adequately. Only a very rough estimate of the sodium exchange rates could be made. Transfer rates for sodium could not be calculated on this basis and more complex multi-compartment models will have to be worked out for this purpose.

APPENDIX

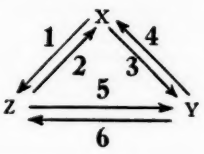
Mathematical analysis of closed steady state two- and three-compartment systems. Since access to all compartments can be assumed, the mathematical treatment of the models is relatively simple. Any combination of transfer rates for a closed three-compartment system can be expressed by the general differential equation:

$$\begin{aligned} \frac{dx}{dt} + ax + by + cz &= 0 \\ \frac{dy}{dt} + a_1x + b_1y + c_1z &= 0 \qquad 5 \\ \frac{dz}{dt} + a_2x + b_2y + c_2z &= 0 \end{aligned}$$

where x, y and z represent the specific activity or concentration of tracer in Compartments X, Y and Z. The coefficients a, b, c, and so forth are related to the transfer rates and their definition depends upon the model under consideration.

For the three-compartment system where X, Y and Z

all exchange:



the nine coefficients are:

$$(1)\quad a = \frac{\rho_1 + \rho_3}{S_x}$$

$$(2)\quad b = \frac{-\rho_4}{S_x}$$

$$(3)\quad c = \frac{-\rho_2}{S_x}$$

$$(4)\quad a_1 = \frac{\rho_3}{S_x}$$

$$(5)\quad b_1 = \frac{\rho_4 + \rho_6}{S_y} \qquad 6$$

$$(6)\quad c_1 = \frac{-\rho_5}{S_y}$$

$$(7)\quad a_2 = \frac{-\rho_1}{S_z}$$

$$(8)\quad b_2 = \frac{-\rho_6}{S_z}$$

$$(9)\quad c_2 = \frac{\rho_2 + \rho_5}{S_z}$$

where the ρ's are the transfer rates in the specific directions defined above and S_x, S_y and S_z the size of Compartments X, Y and Z. The experimental data are given in terms of curves showing the change of x, y and z as a function of time. These curves are the solution of the general differential equation and the treatment of data is concerned with the derivation of coefficients (*1*) to (*9*). The measurable quantities are double exponential equations representing the change of x, y and z with respect to time:

$$\begin{aligned} x &= \alpha_1 e^{r_1 t} + \alpha_2 e^{r_2 t} + \alpha_3 \\ y &= \beta_1 e^{r_1 t} + \beta_2 e^{r_2 t} + \beta_3 \\ z &= \gamma_1 e^{r_1 t} + \gamma_2 e^{r_2 t} + \gamma_3. \end{aligned} \qquad 7$$

Graphic analysis of these curves gives the values for the exponential constants r_1 and r_2 and the integration constants α_1, α_2, β_1, β_2, γ_1 and γ_2. The three constants α_3, β_3 and γ_3 are identical and equal to the equilibrium value in whatever units x, y and z are expressed. The relation of the coefficients and the measurable constants is found by solving the original differential equation. For this solution it is assumed that each variable can be expressed as the sum of one or more exponential terms:

$$x = e^{rt};\quad y = e^{rt};\quad z = e^{rt}.$$

Differentiating each of these terms and substituting in *5* gives:

$$\begin{aligned} (a + r)\alpha + b\beta + c\gamma &= 0 \\ a_1\alpha + (b + r)\beta + c\gamma &= 0 \\ a_2\alpha + b_2\beta + (c_2 + r) &= 0. \end{aligned} \qquad 8$$

There are three integration and three exponential constants, hence nine equations or three sets of the form *8*. From these nine equations the nine coefficients can be calculated or expressed in terms of the measured constants; the following relations are useful for the problem under consideration:

$$a_1 = \frac{\dfrac{-r_1\beta_1}{\beta_1 - \gamma_1} + \dfrac{r_2\beta_2}{\beta_2 - \gamma_2}}{\dfrac{\alpha_1 - \gamma_1}{\beta_1 - \gamma_1} - \dfrac{\alpha_2 - \gamma_2}{\beta_2 - \gamma_2}}$$

$$c_1 = -\frac{r_1\beta_1}{\gamma_1 - \beta_1} - a\frac{\alpha_1 - \beta_1}{\gamma_1 - \beta_1}$$

$$a_2 = \frac{\dfrac{-r_2\gamma_2}{\gamma_2 - \beta_2} + \dfrac{r_1\gamma_1}{\gamma_1 - \beta_1}}{\dfrac{\alpha_1 - \beta_2}{\gamma_2 - \beta_2} - \dfrac{\alpha_1 - \beta_1}{\gamma_1 - \beta_1}} \qquad 9$$

$$c_2 = \frac{-r_1\gamma_1}{\gamma_1 - \beta_1} + a_2\frac{\beta_1 - \alpha_1}{\gamma_1 - \beta_1}$$

$$c = \frac{\dfrac{r_1\alpha_1}{\beta_1 - \alpha_1} - \dfrac{r_2\alpha_2}{\beta_2 - \alpha_2}}{\dfrac{\gamma_2 - \alpha_2}{\beta_2 - \alpha_2} - \dfrac{\gamma_1 - \alpha_1}{\beta_1 - \alpha_1}}.$$

Because of the steady state conditions (the sum of all transfer rates in and out of each compartment is equal to zero) only four of the six coefficients need be calculated directly from the constants, the remaining transfer rates being defined:

$$\begin{aligned} \rho_1 + \rho_6 &= \rho_2 + \rho_5 \\ \rho_2 + \rho_4 &= \rho_1 + \rho_3 \\ \rho_3 + \rho_5 &= \rho_4 + \rho_6. \end{aligned} \qquad 10$$

The analysis of a simplified system can be accomplished in essentially the same manner. If, for example, there is no pathway from mother to amniotic fluid, a catenary system of the type:

$$X \underset{}{\overset{\rho_1}{\rightleftarrows}} Z \overset{\rho_2}{\rightleftarrows} Y$$

represents the kinetic model. With reference to the general differential Equation *5* the coefficients b and a_1 are equal to zero, *i.e.*, the transfer rates ρ_3 and ρ_4 do not exist. Since the system is in a steady state, $a = -c$. Substituting in Equation *5* gives:

$$a\alpha_1 + r\alpha_1 + c\gamma_1 = 0 \qquad 11$$

and

$$c = \frac{r_1\alpha_1}{\gamma_1 - \alpha_1} \quad \text{and} \quad \rho_1 = \frac{-r_1\alpha_1 S_x}{\gamma_1 - \alpha_1}. \qquad 12$$

For the two compartment case, where mother and fetus are "lumped"

$$X \rightleftarrows (Y + Z)$$

the general equation takes the form:

$$\begin{aligned} \frac{dx}{dt} + ax + by &= 0 \\ \frac{dy}{dt} + a_1x + b_1y &= 0. \end{aligned} \qquad 13$$

Differentiating the assumed solutions:

$$x = e^{rt} \quad \text{and} \quad y = e^{rt}$$

and substituting in Equation *13* one obtains:

$$\begin{aligned} (a + r)\alpha_1 + b\beta_1 &= 0 \\ a_1\alpha_1 + (b_1 + r)\beta_1 &= 0 \end{aligned} \qquad 14$$

where the coefficients a, b, a_1 and b_1 are related to the transfer rate

$$a = -b = \frac{\rho}{S_x} \qquad 15$$

$$a_1 = -b_1 = -\frac{\rho}{S_y} \qquad 16$$

$$a\alpha_1 + r\alpha_1 - a\beta = a_1\alpha_1 - a_1\beta_1 + r\beta \qquad 17$$

and

$$\rho = -S_x \frac{r\alpha}{\alpha - \beta} = +S_y \frac{r\beta}{\alpha - \beta} \qquad 18$$

which is identical with the Sheppard and Householder (11) equation since α and β have opposite signs and are proportional to the size of the two compartments.

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THE ROLE OF THE FETUS IN THE WATER EXCHANGE OF THE AMNIOTIC FLUID OF NORMAL AND HYDRAMNIOTIC PATIENTS *

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In a preceding report on exchange mechanisms in pregnant monkeys (1), evidence has been presented that the water exchange between the maternal organism and the products of gestation can be represented by a kinetic model composed of three major compartments, all of which exchange with each other. The three compartments are defined as: *1*) the amniotic fluid, *2*) the maternal, and *3*) the fetal body water. It could be shown that the introduction of a hydrogen tracer into fetus or amniotic fluid results in retention curves of predictable shape and that transfer rates for water can be calculated with reasonable accuracy when the amniotic fluid was used as the primary compartment. It should therefore be possible to devise methods whereby the transfer rates for the human could be calculated and to study the role which the fetus plays in the exchange of water between the amniotic fluid and the mother.

For the calculation of transfer rates it is necessary to know the change in concentration of tracer as a function of time for each compartment. Continuous measurements on pregnant women are limited to the two accessible compartments: mother and amniotic fluid. In addition, a single sample of fetal or cord blood can be obtained at the time of delivery. Under these conditions there are two possible ways in which a time-activity curve for the fetal compartment can be calculated from available data. The first is based upon the fact that, within the relatively brief period of time during which measurements are made, the total amount of tracer in all three compartments is constant. Ideally, the amount of tracer in the fetus should be equal to the difference between the amount of tracer injected and the sum of that in maternal and amniotic fluid compartments. The large difference in size between mother and the other compartments and the evident poor mixing in the maternal and fetal systems renders this approach too inaccurate for practical purposes.

The second possibility of deriving a time-activity curve for the fetus is somewhat more direct and takes advantage of the fact that at least one sample of fetal blood is available at termination of the experiment. The tracer concentration in the fetus follows a double exponential curve for which two points in addition to zero and infinity are necessary and sufficient for definition. If two tracers for hydrogen are injected into the primary compartment (amniotic fluid) at different times, analyses of cord blood for both tracers should give the two points from which the time-activity curve for the fetus can then be reconstructed.

The availability of deuterium and tritium made it possible to apply this method for the measurement of transfer rates in a series of normal pregnant women whose gestational ages ranged from 12 weeks to term. In two additional instances similar measurements were made on hydramniotic patients in an effort to describe further this abnormal state.

THEORY

Two points in addition to zero and infinity define the fetal tracer concentration curve. In theory any two

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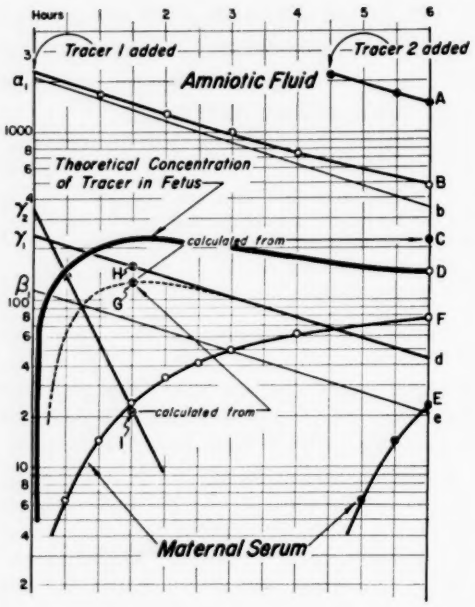

FIG. 1. EXPERIMENTAL DATA OBTAINED ON A HYDRODYNAMIC MODEL (2) ILLUSTRATING THE METHOD OF GRAPHIC ANALYSIS, THE INDIVIDUAL STEPS OF WHICH ARE DESCRIBED IN THE TEXT

The transfer rates and the volume of the three compartments had dimensions in the range anticipated for a term patient. The ordinate represents a scale common to both isotopes based on an estimated equilibrium value of 100 per cent.

points might serve the purpose but the practical application imposes certain limitations. Maximum accuracy would be expected if one of these points were to fall on the ascending, the other on the descending, portion of the curve whose general shape is known. If the fetal tracer concentration curve passes through a maximum, the concentration of the first tracer at any time beyond this maximum will be higher than the equilibrium value, while the concentration of the second tracer may be higher, equal to, or less than that of the first tracer. Under certain circumstances it is possible that the fetal tracer curve does not pass through a maximum, in which case the concentration of the first tracer will always be less than the equilibrium value yet higher than the second tracer. The relative position of these points and the equilibrium value crudely define the shape of the curve.

The manner in which the graphic analysis in all subsequent experiments was carried out is best illustrated by means of an example using a hydrodynamic model previously described (2). The first tracer was added to the "amniotic fluid" compartment at time zero. The concentration of this tracer was then measured in "maternal serum" and "amniotic fluid" every 30 minutes and the results plotted on an exact semilog scale (Figure 1). After four and one-half hours a second tracer was added to the "amniotic fluid" compartment and the experiment terminated at the end of the sixth hour. The tracer concentration for the "amniotic fluid" compartment at that time is given by Points A and B. A sample of fetal blood which had thus far been ignored was now analyzed for the two tracers and yielded Points C and D. The two tracers in the maternal compartment are given by Points E and F. Subtracting the equilibrium value of 100 per cent from each of the experimental points gives a straight line, the first exponential term, for the major portion of the curves. This holds for all three compartments alike. Line $b - \alpha_1$ is the first exponential term for the amniotic fluid and Line $e - \beta_1$ that for the maternal compartment. For the fetal curve this line must be reconstructed from Point D only. D minus 100 gives Point d through which a line parallel to $b - \alpha_1$, the first exponential term, must pass. This line intersects $t = 0$ at γ_1 which determines the first integration constant for the fetal compartment. The value for the second tracer can then be used to determine the second integration constant. C minus 100 gives a Point G which does not fall on Line $d - \gamma_1$ and this difference, the numerical values of H minus G, determines Point I, through which a line representing the second exponential term must pass. Since the tracer concentration for the fetal compartment must be zero at $t = 0$, the second integration constant for this compartment is given by $\gamma_2 = \gamma_1 + 100$. The line

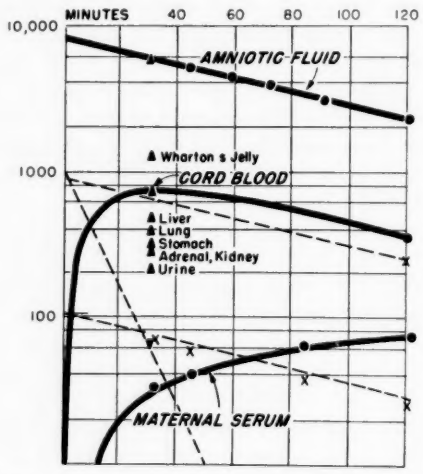

FIG. 2. THE TIME-ACTIVITY CURVES FOR AMNIOTIC FLUID, CORD BLOOD AND MATERNAL SERUM MEASURED ON A PATIENT DURING THE TWENTIETH WEEK OF GESTATION

Deuterium oxide and tritium oxide were injected into the amniotic fluid 120 and 30 minutes *before* hysterotomy. Deuterium analyses are designated by circles; tritium analyses, by upright triangles.

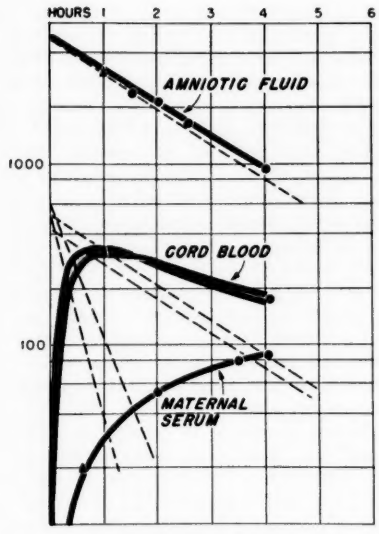

FIG. 3. TIME-ACTIVITY CURVES FOR AMNIOTIC FLUID, CORD BLOOD AND MATERNAL SERUM OBTAINED ON A PATIENT DURING THE TWENTY-NINTH WEEK OF GESTATION

The shaded area between the two curves for cord blood represents the limits compatible with the experimental data. Solid circles are deuterium and upright triangles tritium analyses. The dotted lines are the corresponding limits for the two exponential terms for cord blood.

$\gamma_2 - I$ is the second exponential term for the fetal compartment and its slope the second exponential constant (for all three compartments). The remaining integration constants α_2 and β_2 can be derived from a knowledge of the volume of each compartment and the value of the first integration constant α_1, β_1 and γ_1. These constants are related to the transfer rates in the manner described previously (1).

METHODS

Reference to the analytic procedures for tritium and deuterium has been made in a preceding report (1). Only patients who had obstetrical indications for abdominal cesarean section or hysterotomy were used in this study. The general design of experiments on early and late pregnancies was similar but varied in the technique of the amniotomy and the period of observation over which samples were collected.

In term or near-term patients the amniotomy was done abdominally by a procedure described in detail elsewhere (3). At a predetermined time, ranging from one (normal) to two (hydramnios) hours after the injection of the second tracer, a cesarean section was performed and samples of cord or fetal heart blood obtained.

The four subjects at 12, 15, 15 and 20 weeks had vaginal hysterotomies in accordance with routine policy. Abdominal amniotomy at such early stages of gestation was considered unnecessarily hazardous, hence the procedure was co-ordinated with the subsequent operation. The cervix was grasped with tenacula and, under local anesthesia, a transverse incision was made in the anterior fornix at the junction of cervical and vaginal mucosa. The vesico-cerival space was entered after division of the supra-vaginal septum and the bladder pushed upward by blunt dissection. A 16 gauge needle with stilette was then inserted into the substance of the cervix in the main axis of the fundus at the level of the internal os, the point of entry being just below the anterior peritoneal fold. Upon withdrawal of the stilette a multi-holed polyethylene catheter was introduced and the needle withdrawn over the catheter. The raw edges of muscosa were then approximated with a few interrupted chromic catgut sutures. The catheter, fitted with a two-way stopcock, was taped to the patient's thigh and the experiment was begun.

The general procedure was basically identical for all experiments. On the day before the contemplated operation a total body water determination was carried out by the deuterium oxide dilution method using 50 to 75 ml. of the isotope containing 0.85 per cent sodium chloride. On the following morning an amniotomy was per-

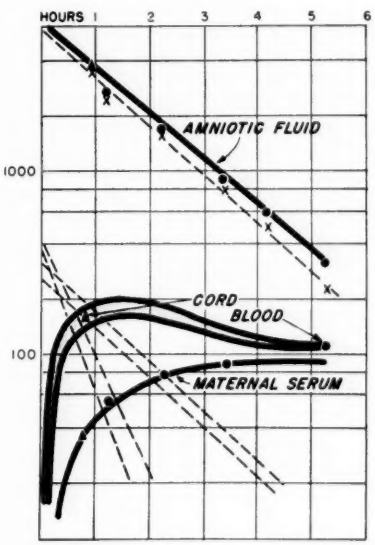

FIG. 4. TIME-ACTIVITY CURVES FOR AMNIOTIC FLUID, CORD BLOOD AND MATERNAL SERUM OBTAINED ON A NORMAL TERM PATIENT SUBJECTED TO ELECTIVE CESAREAN SECTION

Solid circles are deuterium; upright triangles, tritium analyses. The shaded area between the two curves for cord blood represents the limits compatible with experimental data. The dotted lines are the limits of exponential terms for cord blood.

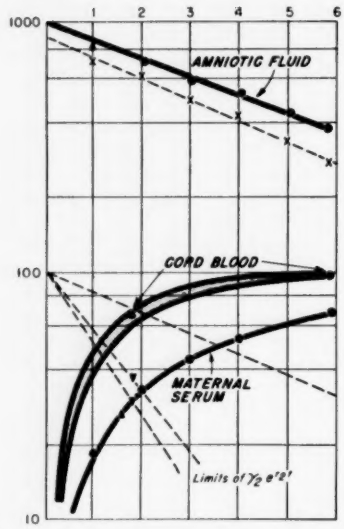

FIG. 5. TIME-ACTIVITY CURVES FOR AMNIOTIC FLUID, CORD BLOOD AND MATERNAL SERUM OBTAINED ON A PATIENT WITH ACUTE HYDRAMNIOS

The shaded area between the two curves for cord blood represents the limits for transfer rates compatible with the experimental data. The dotted lines represent the limits for the two exponential terms.

formed either by the abdominal or vaginal route and control samples of amniotic fluid and maternal blood collected for determination of their deuterium content. A known and accurately measured amount of deuterium oxide was then injected into the amniotic cavity and thoroughly mixed by frequent withdrawal and reinjection of the amniotic fluid with a 20 or 50 ml. syringe. Simultaneously, a known amount of Congo red was introduced into the same compartment for the accurate determination of the fluid volume (4). The withdrawal and reinjection was continued throughout the remainder of the experimental period in order to insure proper mixing and sampling. Amniotic fluid and maternal blood were collected every 15 to 60 minutes. One mc. of tritiated water made isotonic with normal saline was then injected in like manner 20 minutes to two hours before cesarean section or hysterotomy. The interval over which the time-activity curves for the two tracers were determined depended upon the length of gestation and also upon the size of the primary compartment. During the early periods of gestation, hysterotomy was performed 20 to 30 minutes after the injection of tritium, while for the studies on hydramniotic patients at least two hours were allowed to elapse. Cesarean sections and hysterotomies were performed as quickly as possible and samples of cord or fetal heart blood were collected within less than one minute after rupture of the amniotic sac. In the four instances where the operation was performed as a therapeutic termination of pregnancy, samples of the more significant fetal tissues were collected for isotope analyses.

RESULTS

The results on four representative experiments are given in graphic form in Figures 2 through 5. The theoretic tracer concentration curve for the fetus was reconstructed in the manner described for the hydrodynamic model. The integration and exponential constants necessary for the calculations were obtained by graphic analysis of the data as described in the theoretic example

TABLE I

*The calculated transfer rates for water during early pregnancies in ml. per hour**

		No. 2773 (12 weeks)	No. 2726 (15 weeks)	No. 2798 (15 weeks)	No. 2762 (20 weeks)
Fetus to mother	ρ_1	111 (109–114)	259 (121–398)	222 (80–364)	444 (221–667)
Mother to fetus	ρ_2	100 (96–103)	302 (250–354)	256 (195–317)	459 (360–558)
Fetus to A.F.	ρ_3	2.5 (0–5)	70 (0–140)	77 (0–154)	101 (0–202)
A.F. to fetus	ρ_4	11 (10–12)	37 (30–44)	38 (29–47)	78 (64–92)
A.F. to mother	ρ_5	64 (63–65)	107 (85–129)	97 (73–121)	103 (68–139)
Mother to A.F.	ρ_6	72 (70–75)	79 (0–158)	66 (0–132)	89 (0–178)

* The ranges shown represent the highest and lowest possible values compatible with the experimentally determined time-activity curves for the three compartments.

TABLE II

*The transfer rates for water in later stages of gestation and under conditions of hydramnios**

		30 weeks	38 weeks	40 weeks	Hydramnios	Hydramnios
Fetus to mother	ρ_1	1,475 (1,311–1,640)	3,300 (3,180–3,420)	3,682 (3,648–3,716)	423 (420–426)	162 (135–189)
Mother to fetus	ρ_2	1,619 (1,454–1,785)	3,445 (3,295–3,595)	3,657 (3,542–3,772)	881 (878–884)	231 (196–266)
Fetus to A.F.	ρ_3	150 (0–300)	295 (145–445)	149 (0–298)	563 (561–565)	106 (100–112)
A.F. to fetus	ρ_4	164 (157–171)	285 (270–300)	165 (150–174)	99 (97–101)	36 (35–39)
A.F. to mother	ρ_5	160 (153–167)	200 (180–220)	247 (235–259)	527 (524–530)	456 (444–468)
Mother to A.F.	ρ_6	167 (10–324)	189 (34–345)	265 (97–433)	63 (60–66)	387 (383–391)

* The values in parentheses represent the maximal range compatible with measured time-activity curves for the three compartments.

and Figure 1. Table I is a summary of the calculated ranges for transfer rates in early pregnancies and Table II represents the final data for later stages of gestation and hydramniotic patients. The analytic data for the tritium concentration in representative fetal tissues and body fluids are reproduced in Table III. On one set of fetal tissues mass spectrographic deuterium and tritium analyses were carried out in an effort to determine the degree of equilibration which had occurred as a function of time. These findings are reproduced in Table IV. Tritium analyses of specific sections of cord from normal and hydramniotic patients are summarized in Table V.

TABLE III

*Relative tritium concentration in various fetal tissues, urine and placenta 19 to 30 minutes after injection of a known amount of tritium into the amniotic fluid**

Elapsed time (*min.*)	No. 2773 (12 weeks) 19	No. 2726 (15 weeks) 30	No. 2798 (15 weeks) 30	No. 2762 (20 weeks) 30
Wharton's jelly	327	192	171	182
Cord blood	100	100	100	100
Lung	73	65	73	66
Liver		42	53	56
Adrenal			63	40
Kidney	79		65	37
C.S.F.	62	53		
Stomach		49	58	45
Urine		35		31
Placenta	28	47	58	45

* The values were obtained by combustion of wet tissue and the specific activity is related to cord blood, arbitrarily set at 100 per cent.

DISCUSSION

In addition to the main assumptions upon which all tracer experiments are based, the system here considered must be in a steady state and the two tracers must perform in identical manner at least over the brief period of observation. Because of the wide differences in the mass of hydrogen, deuterium and tritium, an isotope effect could conceivably occur. In order to demonstrate that the assumption of identical performance is valid, a series of experiments was carried out in which first deuterium and then tritium was injected into the amniotic fluid of pregnant patients at term. Their disappearance from the amniotic fluid and appearance in the

TABLE IV

*Hydrogen isotope concentrations in fetal tissues obtained on Patient 2762 (Figure 2, Table III)**

	Tritium	Deuterium
Wharton's jelly	182	
Cord blood	100	100
Lung	66	72
Liver	56	78
Adrenal	40	60
Kidney	37	49
Stomach	45	50
Placenta	45	48
Urine	31	70

* For purposes of comparison the concentration of each isotope in cord blood was arbitrarily set at 100. The tritium values were obtained 30 minutes, the deuterium values 120 minutes, after the injection of the isotope.

TABLE V

Comparison of the tritium concentration in cord blood and Wharton's jelly one to two hours after injection of a known amount of tritium into the amniotic sac *

	Cord serum	Cord near fetus	Cord near placenta
Normal (30 weeks)	330	1,095	711
Normal (term)	160	1,138	875
Hydramnios† (term)	26	166	139
Hydramnios‡ (term)	68	248	194

* All values are referred to an equilibrium value of 100 per cent.
† Normal baby.
‡ Congenitally abnormal baby.

maternal circulation was measured. It could be shown that in the same patient the two tracers give identical time-activity curves. The results of one such experiment are reproduced in Figure 6, which shows how the two tracer curves "chase" each other, the second (tritium) reproducing a pattern identical with the first (deuterium), temporally displaced. An isotope effect, if it occurs at all, is therefore negligible and is not expected to have an appreciable influence upon the final results.

Qualitatively, the shape of the retention and appearance curves for the three compartments is predictable. For the calculation of transfer rates, they must be determined with the highest possible accuracy and only the most reliable sections of these curves can be considered. The time interval from injection of the two tracers to the termination of the experiment must be so chosen that the two points for the fetal curve fall within a specific range: the first tracer on the descending and the second tracer on the ascending portion of the curve. In the experiments carried out during early pregnancies only a very rough estimate could be made on the basis of models and previous experience on term patients. Slightly more reliable curves than those shown in Figure 2 could have been obtained had the experiments been carried out over a longer period of time and the exposure to tritium shortened. Tritium exposures of less than 20 minutes would give experimental points on the rising portion of the curve and thus, at least in theory, lead to a more accurate calculation of the second exponential constant. Unfortunately, such brief intervals probably would not permit an adequate distribution of the tracer within this compartment and the tritium concentration then would not be comparable to the deuterium concentration in the same sample of cord blood.

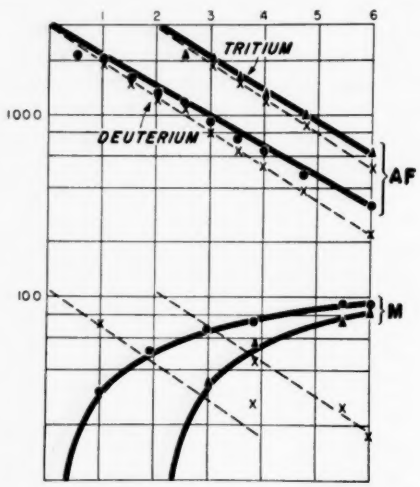

FIG. 6. DEUTERIUM AND TRITIUM CONCENTRATION IN AMNIOTIC FLUID (A.F.) AND MOTHER (M) AS A FUNCTION OF TIME IN HOURS

Both tracers were injected into the amniotic sac at the time indicated.

The functional anatomic complexity of the maternal organism leads to unreliable tracer curves for this compartment which cannot be used for the calculation of transfer rates. Attempts to do so in experiments carried out on rhesus monkeys (1) led to inconsistent results. Fortunately, it is not necessary to use the maternal curves for this purpose since all the essential information can be obtained from the values of the amniotic fluid and fetal compartment.

Satisfactory mixing in the amniotic fluid compartment is evident from its consistent retention curves. Whenever the amniotic fluid volume was large in comparison to the size of the fetus (*e.g.*, in early pregnancies and under conditions of hydramnios) the first exponential constant could be fixed within very narrow limits. Only an exceedingly small error was therefore carried over to the second exponential constant which, in turn, depends upon the accuracy of the deuterium analysis in cord blood. The analytic error in these determinations can be brought to negligible proportions and the total error depends mainly upon the degree of mixing in the fetal compartment.

The analyses of tissue samples made it possible to estimate the extent of distribution which had taken place since the injection of both tracers. The analytic data of Table III were obtained by combustion of wet tissues rather than lyophilization; hence, the specific activity of the hydrogen gas produced requires correction. In the combustion of solid fetal tissues unlabeled hydrogen is introduced. This dilution can be estimated as about 20 to 25 per cent if it is assumed that the sample consisted of not more than 40 per cent solids. The actual isotope concentration is, therefore, considerably higher than would appear from the data of Table III and if such corrections were made, the data would indicate a nearly complete equilibration within the brief period of 20 to 30 minutes. With or without this correction, the highest specific activity was found in lung and there was a relatively even distribution in the other organs. The isotope concentration in placental tissue was invariably low while Wharton's jelly had a relative specific activity which exceeded that of cord blood several times. Comparing the distribution of tritium with the distribution of deuterium in the same subject and the same samples of hydrogen gas obtained by combustion (Table IV) it is evident that there was only a very slight, relative increase of the tracer concentration over an additional 120 minutes of exposure. The only exception appears to be the sharp rise of isotope content in fetal urine (from 31 to 70 per cent) which represents definite evidence that the fetal kidneys are active, even at 12 weeks of gestation.

As pregnancy progresses the role of the fetus in the various exchange mechanisms might possibly change. At 12 weeks gestation the exchange between amiotic fluid and fetus is small compared to the direct exchange between amniotic fluid and mother. At term 40 per cent of the transfer from the amniotic fluid to the maternal system takes place through the intermedium of the fetus. This is not at variance with indirect evidence previously presented (5) that at term at least 25 per cent of the water transfer from the amniotic fluid to mother takes place through the fetus. The reported transfer rates are given in terms of a range compatible with the measured experimental data. Even the lowest possible values for the transfer of water from mother to

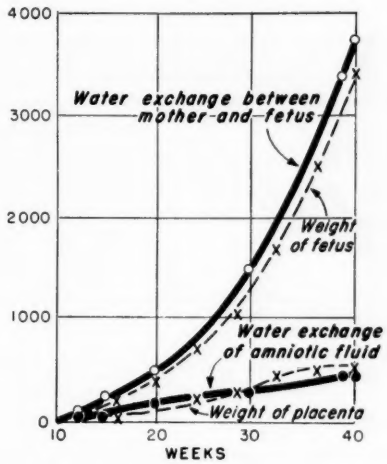

FIG. 7. THE RELATION OF WATER EXCHANGE IN ML. PER HOUR TO AGE OF GESTATION

The values are related to reported average values for fetal and placental weight in grams.

amniotic fluid constitute an appreciable proportion of the total exchange and hence this pathway exists throughout pregnancy.

The transport of water from mother to fetus, presumably via the placenta, has been investigated by Hellman and co-workers (6), who found a progressive rise with age of gestation followed by a sharp decline at term. This was attributed to aging of the placenta, a phenomenon correlated with morphologic (7) and biochemical studies (8). In the present investigations this decline in water transmission near term could not be confirmed and the measurements point to a constant rise in this exchange rate until delivery as shown in Figure 7. The amount of water exchanged per hour increases from about 100 ml. per hour during the twelfth week to 3,600 ml. per hour at term. A similar, consistent rise in the total water exchange of the amniotic fluid has been observed which, incidentally, appears to be proportional to the increase in placental weight. Under the pathologic condition of hydramnios the water exchange between the maternal and fetal organism is grossly impaired. As shown in Table II this exchange rate in one of the patients was about one to eight, in the other less than one to 20 that of normal pregnant women. The difference in these transfer rates is beyond the

TABLE VI

The total transfer of water, sodium and potassium from amniotic fluid to mother and fetus in normal and hydramniotic patients *

A. F. volume	Transfer rates (*mEq./hr.*)		
	D_2O	Na	K
Less than 2,000 ml. (range, 385–1,420)	26,100	12.0	0.5
More than 2,000 ml. (range, 2,000–6,000)	27,300	12.3	0.6

* There is no statistically significant difference between the two groups of patients.

experimental error and the relatively narrow limits for these measurements render the data very reliable.

The transfer from amniotic fluid directly to the mother (ρ_5) is proportionately increased, thus superficially resembling the situation prevailing during earlier periods of gestation. The total amount of water leaving the amniotic fluid per unit of time (ρ_4 plus ρ_5) for both groups is not appreciably different for the two groups. It amounts to 492 and 626 ml. per hour for the two hydramniotic patients and 412 and 490 ml. per hour for the two normal term patients. This confirms the earlier observations of Hutchinson, Hunter, Neslen and Plentl (9) that the total water and electrolyte transfer is independent of the volume of the amniotic fluid. For comparison, a summary of the data reported by these authors is given in Table VI. In hydramniotic patients, therefore, the amount of water leaving the amniotic fluid compartment is about the same or only slightly increased over that measured in normal patients. Thus, the "removal" of water is certainly not impaired; if anything, it is increased. The two groups, however, differ in one important aspect: In the normal patient most of this transfer is accomplished through the intermedium of the fetus, while under conditions of hydramnios a by-pass of some sort seems to exist. This may be a simple diffusion phenomenon directly transmitting the water from the amniotic fluid to the maternal organism or more likely related to some change or lesion of the feto-placental circulatory system. That some physiologic, functional change has occurred in this system is shown by the depressed exchange of water between mother and fetus (Figure 8).

In the experiments on term pregnancies samples of fetal tissue were not available, but sections of placentae and cord were examined for their tritium content. The tracer concentration in placental tissue was less than that of cord blood and did not show any appreciable variations in samples taken from different areas of this organ. Wharton's jelly, on the other hand, showed a consistent gradient. The specific activity of samples taken near the baby was invariably higher than that of cord samples near the placental insertion. The difference was appreciable, consistent, and beyond the experimental error of the method. The significance of this gradient is not clear at the present time but may well be related to a specific transfer mechanism of water through the cord.

With certain reservations these data might be used for tentative conclusions regarding the sites of exchange. The strikingly high concentration of tritium in Wharton's jelly makes it very likely that the major part, perhaps all, of the water exchange of the amniotic fluid takes place through this organ. A direct transfer through the membranes and decidua to the maternal system cannot be ruled out and the data obtained in this investigation cannot be used to distinguish between these possibilities. The low specific activity of placental tissue when compared to cord blood does not permit similar conclusions to be drawn since the transmission of water across the placenta is a rapid process and the appearance of the tracer a transitory phenomenon. It is to be expected that the specific activity of placental tissue be less than cord blood yet more than that of maternal serum.

The site and mechanisms of water exchange between amniotic fluid and fetus are not entirely clear. In these experiments the amniotic fluid was the primary compartment and its tracer concentration was appreciably higher than that of the other two compartments at any time during the experimental period. Stomach and lung, if contaminated with amniotic fluid, should contain very appreciable amounts of tritium in excess of that transmitted through the fetal blood stream. Although there are considerable variations from one specimen to another, the findings were quite consistent for the tissues obtained during the early periods of gestation. None of them, except Wharton's jelly, had an isotope concentration greater than that of cord blood. Swallowing and aspira-

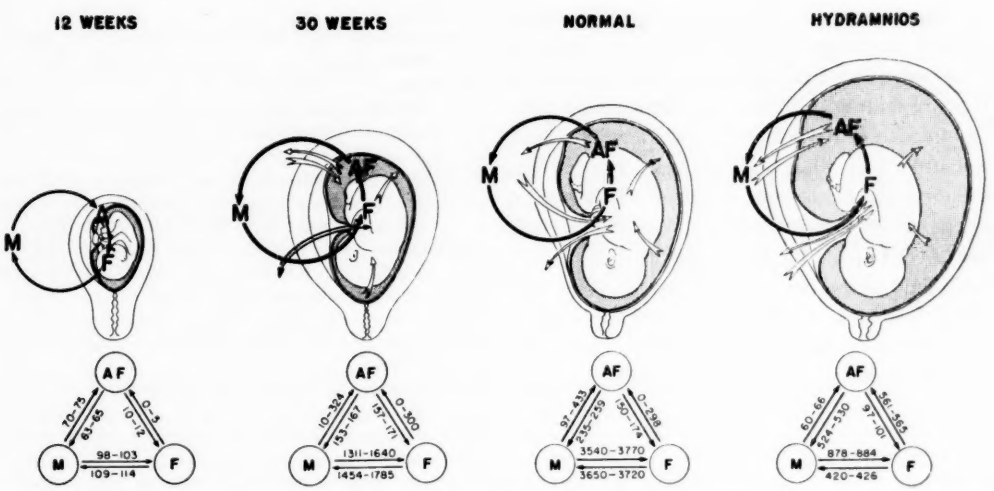

FIG. 8. SCHEMATIC PRESENTATION OF THE WATER EXCHANGE BETWEEN MOTHER (M), FETUS (F) AND AMNIOTIC FLUID (A.F.) IN NORMAL AND PATHOLOGIC PREGNANCIES

The arrows in the lower portion of the diagram indicate the direction; the values assigned to them, the hourly transfer in milliliters. The heavy circles to the left of each diagram designate the direction of the net transfer, *i.e.*, the "circulation" of the water.

tion may well account for a small part of the transfer between amniotic fluid and fetus but it is likely that most of the water passes through the cord.

SUMMARY

The quantity of water transferred between amniotic fluid, fetus and mother has been determined by means of isotopic tracers. Because of the inaccessibility of the human fetus, time-activity curves for the fetal compartment cannot be determined directly and a method was devised and tested whereby this curve could be reconstructed from the amniotic fluid retention curve and a single sample of cord blood obtained at the time of delivery.

2. The method consists of injecting first a known amount of deuterium oxide followed by tritium oxide into the amniotic sac of pregnant women. Fifteen to 60 minutes after the injection of the second tracer cesarean sections or hysterotomies were performed. The concentration of the two tracers in cord blood, in addition to the initial and final, theoretic equilibrium value, define the fetal curve. The mathematical treatment of the data made it possible to calculate the six transfer rates between the three hypothetical compartments.

3. Seven such experiments were carried out on normal pregnant women whose gestational ages ranged from 12 weeks to term. In the early stages of pregnancy the exchange of water between amniotic fluid and mother is of about the same magnitude as the exchange between mother and fetus. As pregnancy progresses the role of the fetus in this transfer becomes more significant and at term about 40 per cent of the water transfer from amniotic fluid to mother is accomplished through the intermedium of the fetus. Throughout the entire period of gestation there exists a direct pathway of exchange between amniotic fluid and maternal organism. The exchange of water between mother and fetus rises progressively with fetal weight and amounts to 3.5 L. per hour at term.

4. Identical studies carried out on hydramniotic patients at term led to the conclusion that this pathologic state is associated with a pronounced decline in the exchange rate between mother and fetus. Most of the water of the amniotic fluid now originates from the fetal system and most of its removal is accomplished by a direct transfer to the maternal compartment.

5. Deuterium and tritium analyses of organs obtained from four nonviable fetuses indicated an almost complete equilibration within a period of 20 to 30 minutes. Thirty and 120 minutes after the injection of the tracers into the amniotic fluid, fetal urine had 30 and 70 per cent, respectively, of the cord blood levels. Wharton's jelly had an isotope concentration several times higher than that of cord blood, but lower than that of the amniotic fluid at the time of termination. This consistent finding was thought to indicate that most, perhaps all, of the water transfer between amniotic fluid and fetus takes place through the cord.

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PROCEEDINGS OF THE FIFTY-FIRST ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N. J., MAY 4, 1959

PRESIDENTIAL ADDRESS

OBSERVATIONS ON GROWTH AND DEVELOPMENT OF CLINICAL INVESTIGATION

BY JOHN A. LUETSCHER, JR.

With this meeting, our Society begins its second half-century. Fifty years ago, the first president, Dr. S. J. Meltzer, addressed the 15 members present at the first meeting of the Society. His topic was, "The growth and differentiation of the medical sciences," a subject of lasting interest and importance. "In years gone by, medicine was a unit and its leaders tried to master all its aspects. With the development of scientific methods and the growth of knowledge, heavy branches grew out of the stem of medicine, broke off and obtained an independent existence. Anatomy broke away early, then followed physiology, pathologic anatomy, pharmacology, physiologic chemistry and bacteriology. What is left of the old stem is clinical medicine—but this is made up of one part which is the practice of medicine, an applied science which has many elements of an art, and the other part is clinical investigation, which ought to be coordinate to the other pure sciences. The men to carry on this research must not only be informed and trained in the other sciences of medicine, but they must have carried on investigations in pure science, to learn to shape a problem so as to make it amenable to a solution, to marshal the steps of investigation to answer the question, to avoid bias in the search, to apply criticism to the findings, to trust few facts, to temper enthusiasm over discovery, and not to be disheartened by failure. Thus they acquire the habits and tastes of the scientist and investigator. However, after all these preparations, they must select clinical research as the main field of their scientific activity. Teaching medicine and furthering its science is a serious business which ought to be carried on by men who are ready to devote all (or most) of their time to it."

The constitution proposed by Dr. Meltzer, Dr. Warfield Longcope and Dr. Henry Christian recapitulated these broad objectives: "The cultivation of clinical research by the methods of the natural sciences; the unification of science and practice of medicine; the encouragement of scientific investigation by the practitioner; the diffusion of a scientific spirit among its members and among the students who come under their charge."

At first, the Society grew in a typical exponential curve. Within 10 years, it was necessary to limit the program. By 1924, the membership was restricted, but at the same time provision was made for transferring older members to emeritus status, thus opening up places for younger men and ensuring their predominance in the affairs of the Society. Economic depression or war may have limited university appointments or opportunities for research, but they sharpened the insight and determination of many young physicians to fulfil their ideals in clinical research. More recently, with active encouragement, the field of clinical investigation has entered another stage of explosive growth. The members return faithfully to the annual rites of the Society to find the temple crowded (if not outgrown), the reports of investigations more penetrating and significant (but also more specialized) and their officers bewailing travails of choosing, from a great wealth of deserving candidates and brilliant works, a few individuals and papers to fill the quotas set by the Society.

Growth is normal and inevitable in a favorable environment. Populations increase whenever opportunity for expansion is offered to a healthy and vigorous stock, when land and livelihood can be had for a reasonable effort. Growth slows and stops when opportunity ends, when crowding, poverty, discouragement, or disease become prevalent. On the other hand, rapid population expansion may appear again when one factor (such as health) is improved—and although the cause is admirable, the result may be a serious disturbance of established ecologic relationships.

The present rapid growth of clinical investigation is attributable to a substantial increase in gifts and support, translated into fruitful activity by clinical investigators in our medical schools and hospitals. This is no temporary spurt. Barring war or unforeseen disaster, public support for clinical investigation will continue as long as the investigators deserve it. The public rightly believe that their future depends on scientific research, and they take a personal and detailed interest in medical research. Clinical investigators are obligated to maintain such high standards that no general disappointment and disillusionment will cast shadows across their future. The public is reasonably tolerant of premature and extravagant claims, but each retreat entails a perceptible reduction in the audience's confidence in the proponent. Publicity is poorly correlated with significance, and too much outside attention may interfere with the conduct of work. The dignity and integrity of scientific publication are of concern to every scientist.

Increasing the mass of clinical investigation will not necessarily produce a proportional increase in while

results. Elaborate projects, which attempt by sheer size to explore every possible contingency, generally produce more data than enlightenment. New ideas and contributions are likely to come from the investigator with the training and attitudes which Dr. Meltzer set forth, working in the laboratory and clinic with enough help to save him from routine chores, but not so many assistants as to insulate him from sensitive contact with the patients or the tests and measurements upon which his research depends. If clinical research is to prosper, more investigators must be developed, and suitable posts must be established for them.

Clinical investigation has somewhat more complex growth requirements than research grants alone will satisfy. Twenty years ago, Dr. Alan Gregg of the Rockefeller Foundation wrote, "Short-term grants buy most for the dollar, but violate sentiment and lead to resentment. Lots of little grants build up paper work; investigative work loses its tempo; worry about renewal diverts the investigator toward easy goals; junior staff become suspicious or embittered; administrators are uneasy." The influx of large research funds and their additional staff has been most welcome; but to prepare for growth, not only more students and fellows but also more faculty and more space are required in our medical schools.

Full-time faculty must be increased if we are to increase our pace of teaching, seeking out and cultivating new talent, research and administration. Differentiation of functions and delegation of duties offer temporary relief, but beyond a certain point, Parkinson's Law comes into play. Imagine, if you can, the present university faculty with ever-increasing loads of teaching, research, patient-care and administration; surrounded by large numbers of talented young men in training for a few permanent posts; all men being appointed for short periods and their work supported on short-lived grants. Now imagine the professor-administrator teetering on the apex of this unstable human pyramid. E. Northcote Parkinson has given the agitated paralysis of James Parkinson a whole new dimension. Let us give thanks for present blessings, but let us also encourage and support those who work hopefully toward solutions of remaining problems.

What will be the effects of the growth of clinical investigation on this Society? The membership of the Society will increase gradually under the present plan, and further increases will be proposed if the present trend continues. Increasing numbers of candidates will cause more sleepless nights for the Council, and some changes in the administration of the Society's affairs may become necessary. It is to be hoped that sufficient places will be made available so that the Society will never appear as a remote and unattainable goal to the promising young investigator. If the doorway to admission is too widely opened, however, membership will quickly rise above a manageable functional limit. Undue corpulence reduces the mobility, efficiency and prospects for survival of an adult. A more natural expression of growth after maturity is the appearance of new members of the species. In a well-regulated family, the younger members pass through stages of progressively increasing independence and occasional healthy antagonism; but sentiment joins with mutual interest and advantage to hold the group together. The traditional meeting of this Society on the day before the Association of American Physicians has reflected a long and happy community of interest. We welcome the opportunity to join with the American Federation of Clinical Research in sponsoring the Section meetings.

If the Society is to progress toward the promise and goals of its founders, their lively pioneer spirit must be neither drowned in excessive numbers nor immobilized in outdated tradition. Let us look to the next 50 years with a welcome to many more of Dr. Meltzer's "brainy young men" and to their fresh ideas and new programs for this ever youthful Society.

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PAPERS PRESENTED AT THE FIFTY-FIRST ANNUAL MEETING 1959

1. Detection of Pulmonary Arteriovenous Shunts Using Intravenous Injections of Kr^{85} and T-1824 Dye. HARRY W. FRITTS, JR., ALFRED HARDEWIG, DUDLEY ROCHESTER, and JACQUES DURAND, New York, N. Y. (Introduced by Dickinson W. Richards). (1006)
2. The Modification of Myocardial Blood Flow and Oxygen Consumption During Postprandial Lipemia and Heparin-Induced Lipolysis. TIMOTHY J. REGAN, KENAN BINAK, SEYMOUR GORDON, VALENTINO DEFAZIO, and HARPER K. HELLEMS,* Detroit, Michigan. (1033)
3. The Mechanism of Venous Pressure Elevation with Exercise in Human Congestive Heart Failure. J. EDWIN WOOD, Augusta, Ga. (Introduced by Thomas Findley). (1055)
4. Studies on the Blood-Cerebrospinal Fluid Barrier in Man. DAVID P. RALL, EDWARD MOORE, NATHAN TAYLOR, and CHARLES G. ZUBROD,* Bethesda, Md. (1032)
5. Renal Autoexplanation and a Functional Characterization of Non-Excretory Renal Tissue. E. E. MUIRHEAD, J. A. STIRMAN, and FRANCES JONES, Dallas, Texas. (1027)
6. An Analysis of the Intra-renal Mechanisms for Sodium Excretion in the Diseased Kidney Employing an Experimental Model with One Normal and One Diseased Kidney. NEAL S. BRICKER,* PETER A. F. MORRIN, S. WESLEY KIME, JR., RAYMOND G. SCHULTZE, and CLAIRE KLEIN, St. Louis, Missouri. (990)
7. Functional Disparity Between the Two Kidneys in Essential Hypertension. DAVID S. BALDWIN, WILLIAM H. HULET, ERVIN A. GOMBOS, and ALBERT W. BIGGS, New York, N. Y. (Introduced by Herbert Chasis). (985)
8. Organic Acids in Body Fluids of the Uremic Patient. D. SELIGSON, L. W. BLUEMLE, JR., G. D. WEBSTER, JR., and D. SENESKY, Philadelphia, Pa. (Introduced by W. C. Stadie). (1042)
9. Magnesium Deficiency Tetany in Man. DAVID D. ULMER, WARREN E. C. WACKER and BERT L. VALLEE,* Boston, Mass. (1049)
10. Detection of the Heterozygous Carrier of the Wilson's Disease Gene. IRMIN STERNLIEB, ANATOL G. MORELL, and I. HERBERT SCHEINBERG,* New York, N. Y. (1046)
11. Biochemical Studies and Specific Therapy in Hepatic Glycogen Storage Disease. C. U. LOWE,* J. E. SOKAL, B. H. DORAY, and E. J. SARCIONE, Buffalo, N. Y. (1021)
12. Chemical and Clinical Studies of Bromsulfalein (BSP) Metabolites. JOHN V. CARBONE, G. M. GRODSKY, and R. FANSKA, San Francisco, Calif. (Introduced by Maurice Sokolow). (994)
13. The Transport and Synthesis of Lipid by the Small Intestine. ANTHONY M. DAWSON, VIRGINIA M. BELL, and KURT J. ISSELBACHER, Boston, Mass. (Introduced by Marian W. Ropes). (999)
14. The Characteristic Pattern of Esophageal Dysfunction Due to Hiatal Hernia Demonstrated by Fluorocinematography and Simultaneous Pressure Recording. E. CLINTON TEXTER, JR., HAROLD P. LAZAR, ERNESTO J. PULETTI, Chicago, Illinois, and GASTON VANTRAPPEN, Louvain, Belgium. (Introduced by David P. Earle). (1048)
15. A New Clinical Entity in Patients Adrenalectomized for Cushing's Syndrome. DON H. NELSON * and J. WILLIAM MEAKIN, Boston, Mass. (1028)
16. Alterations in the Plasma Transport of Thyroxine in Sick Patients and Their Relation to the Abnormality in Graves' Disease. JOHN B. RICHARDS, J. THOMAS DOWLING, and SIDNEY H. INGBAR,* Boston, Mass. (1035)
17. The Hypocholesteremic Effect of Androsterone. LEON HELLMAN,* H. LEON BRADLOW, BARNETT ZUMOFF, DAVID K. FUKUSHIMA, and T. F. GALLAGHER, New York, N. Y. (1010)
18. The Fatty Acid Composition of Adipose Tissue in Man. JULES HIRSCH, JOHN W. FARQUHAR, MALCOLM L. PETERSON, and WILHELM STOFFEL, New York, (Introduced by E. H. Ahrens, Jr.). (1011)
19. Effect of Insulin and Anaerobiosis on the Membrane Transport and Intracellular Metabolism of Glucose in Diabetic Muscle. H. E. MORGAN, D. M. REGEN, and C. R. PARK,* Nashville, Tenn. (1026)
20. Hyperpolarization of Muscle Induced by Insulin in the Absence of Glucose. KENNETH L. ZIERLER,* Baltimore, Md. (1057)
21. Pathogenesis of the Hemorrhagic Diathesis Developing During "Fibrinolytic" States: The Significance of Defective Fibrin Polymerization. ANTHONY P. FLETCHER,* NORMA ALKJAERSIG, and SOL SHERRY,* St. Louis, Mo. (1005)
22. The Relationship between Genotype and $Rh_o(D)$ Antigen Sites on the Red Blood Cell. S. P. Masouredis, Pittsburgh, Pa. (Introduced by Jonas E. Salk). (1024)
23. Pyrimidine Studies in Pernicious Anemia. LLOYD H. SMITH, JR., and FAITH BAKER, Boston, Mass. (Introduced by Edward Bland). (1044)
24. Treatment of Acute Leukemia by Supra-Lethal Whole-Body Irradiation and Isologous Marrow Transplantation. E. DONNALL THOMAS,* HARRY L. LOCHTE, JR., and OTTO D. SAHLER, Cooperstown, N. Y. (1048)
25. The Effect of X-radiation upon Delayed Hypersensitivity and Antibody Formation in Guinea Pigs. JONATHAN W. UHR and MATTHEW SCHARFF, New York, N. Y. (Introduced by Lewis Thomas). (1049)
26. Inhibition of Virus Multiplication by Transferrin. CHRISTOPHER M. MARTIN and JAMES H. JANDL,* Boston, Mass. (1024)
27. Observations on the Course of Chronic Non-Obstructed Pyelonephritis in the Rat. LUCIEN B. GUZE, BERNARD H. GOLDNER, SYDNEY FINEGOLD, and WILLIAM HEWITT, Los Angeles, California. (Introduced by Samuel H. Bassett). (1009)

* Member.

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ABSTRACTS

Metabolism of L-Triiodothyronine by Human Mitochondria. EDWIN C. ALBRIGHT,* KENKICHI TOMITA and FRANK C. LARSON, Madison, Wisc.

Mitochondria prepared from rat kidney, liver and heart have been found to contain an enzyme system which converts L-triiodothyronine to triiodothyroacetic acid. The present study extends this observation to the human.

Samples of normal liver and kidney were obtained at operation. Mitochondria, isolated by the method of Schneider, were incubated for two hours at 37° C. in a reaction mixture containing phosphate buffer (pH 7.4), DPN and 0.01 μg. of I^{131} labeled L-triiodothyronine. Mitochondria which had been boiled for 10 minutes were used as controls. The incubated reaction mixtures were extracted with 10 volumes of ammoniacal butanol. After concentration and the addition of marker triiodothyroacetic acid the extracts were chromatographed in descent using tertiary amyl alcohol saturated with 2 N NH_4OH. The radioactive compounds were located with a scanner. The position of the marker triiodothyroacetic acid was determined by color development with 4-amino-antipyrine.

Conversion of L-triiodothyronine to triiodothyroacetic acid was observed with both liver and kidney mitochondria. The liver mitochondria appeared to be more active on the basis of per cent substrate converted per milligram of enzyme nitrogen.

Differentiation of a Chloroform-Induced Esterase of Human Serum from Plasmin and its Possible Identity with the First Component of Complement. FRANK K. AUSTEN, Boston, Mass. (Introduced by Henry K. Beecher).

Plasmin (streptokinase-activated serum) is fibrinolytic and caseinolytic, and can act as an esterase capable of splitting *p*-toluene L-arginine methyl ester (TAMe). Chloroform-activated serum is also fibrinolytic and has been considered by Christensen and MacLeod to be identical with plasmin. To evaluate this assumption a comparison of the esterase activity of human serum activated with chloroform and streptokinase was undertaken.

The chloroform-induced esterase differed from plasmin in five ways: *1*) The proenzyme of the chloroform-induced esterase is destroyed by heating serum at 56° for 30 minutes whereas plasminogen is stable. *2*) The chloroform-induced esterase is not inhibited by soybean trypsin inhibitor (SBI) whereas plasmin is. *3*) The chloroform-induced esterase splits N-acetyl L-tyrosine ethyl ester (ATEe); plasmin does not. *4*) Plasmin splits casein but chloroform-activated serum does not, even when the TAMe esterase activity of the two preparations is identical. *5*) Pretreatment of serum with an antigen-antibody precipitate greatly diminishes esterase activity resulting from chloroform activation but does not reduce that from streptokinase activation. Furthermore, removal of the proenzyme of the chloroform-induced esterase from serum is associated with the appearance of TAMe and ATEe esterase activity on the antigen-antibody precipitate.

These observations indicate that the chloroform-induced esterase is different from plasmin. Lepow, Ratnoff and Pillemer demonstrated that an esterase could be eluted from antigen-antibody precipitates pretreated with human serum; evidence was presented that this eluate which splits TAMe and ATEe but not casein and is resistant to SBI represents the activated first component of complement. The similarity between this eluate and the chloroform-induced esterase suggests that chloroform activates the first component of complement.

Veno-Arterial Perfusion as Therapy for the Failing Heart. MARVIN BACANER, JOHN CONNELLY and DAVID BRUNS, San Francisco and Berkeley, Cal. (Introduced by John H. Lawrence).

Fifty-four experiments were performed to quantitate the influence of coronary blood flow (CBF) upon performance of the *in situ* failing dog heart. Heart failure was induced by progressive pulmonary artery constriction (PAC) until systemic blood pressure, cardiac output, CBF and left ventricular stroke work (LVSW) were significantly reduced.

After establishing failure, the coronary arteries were perfused (PAC unaltered) by shunting venous blood to a femoral artery by pump, or by constricting the thoracic aorta.

By external counting over exposed heart: *1*) Relative CBF was determined by disappearance rate (T½) of 0.1 ml. Na^{22} injected into the left ventricular myocardium. *2*) Cardiac output was measured by arterial dilution curve of I^{131} rose bengal. Data were programmed for an IBM 650 digital computer to calculate LVSW and myocardial power.

During failure LVSW decreased, often to ventricular standstill, with concomitant decrease in CBF. Coronary perfusion by either method was invariably accompanied by dramatic changes: *1*) The markedly dilated, feebly contracting, occasionally asystolic heart rapidly decreased in size and began contracting vigorously. *2*) The Na^{22} T½ indicated a marked increase in CBF associated with a striking increase in LVSW which could be maintained by perfusion. Upon stopping perfusion the heart rapidly failed, but improved when perfusion was restarted. *3*) About one time in five, cardiac performance remained well sustained after ceasing perfusion (despite unchanged PAC) indicating an acquired capacity to adjust to work loads that were disabling when CBF was inadequate.

* Member.

4) Rarely (5 per cent), when PAC was extreme, LVSW increased transiently, then fell again despite perfusion.

These results demonstrate a crucial relationship between CBF and cardiac performance and indicate that contractile power, diastolic volume and adaptive mechanisms to increased loads are critically influenced by CBF. The veno-arterial shunt procedure is under clinical trial in myocardial failure and shock where increasing CBF might improve cardiac performance.

Platelet Survival and Viability of Stored Platelets. MARIO BALDINI, NICHOLAS COSTEA and WALTER SMALL, Boston, Mass. (Introduced by Samuel Proger).

Since viability of platelets has importance for their effectiveness in improving hemostasis, studies were performed on the survival of transfused human platelets using the *in vitro* labeling technique with $Na_2Cr^{51}O_4$.

It was first found that two factors have to be considered in the evaluation of the survival of infused labeled platelets: *1*) the yield of radioactive platelets in the recipient, and *2*) the platelet life span.

With the method used, the recovered platelet radioactivity in normal recipients averaged 45 per cent of the injected amount. The average platelet survival time was nine days. The loss of platelet radioactivity in the circulation was linear. *In vitro* as well as *in vivo* studies showed no elution of label from the platelets.

Platelets from patients with thrombocytosis showed a shorter than normal survival with exponential disappearance, both in normal recipients as well as in the patients themselves.

Excessive manipulation of platelets either reduced their yield in the recipient, or their life span, or both.

When reduced amounts of anticoagulant (EDTA) were used, the yield of platelets in the recipient increased. There was no effect on the survival time.

Excess of triton and also excess of metallic chromium reduced both the yield and the life span of the platelets.

The effect of a short term storage on platelet viability was studied. Platelets stored for 24 hours at 4° C. showed a short survival and disappeared from the circulation in exponential fashion. When stored in whole blood the yield in the recipient was in the low normal range, but the mean survival time was 34 hours. Platelets stored in plasma showed no significant reduction of yield in the recipient, but survived 28 hours. Platelets stored in gelatin showed a yield of only 15 per cent of the normal and disappeared from the circulation in 15 hours.

Functional Disparity Between the Two Kidneys in Essential Hypertension. DAVID S. BALDWIN, WILLIAM H. HULET, ERVIN A. GOMBOS and ALBERT W. BIGGS, New York, N. Y. (Introduced by Herbert Chasis).

A renal basis for essential hypertension would be favored by demonstration of functional abnormality early in the disease. Though the causal defect may be bilateral, the only feasible approach appears to be the demonstration of unilateral renal impairment in hypertensive patients without gross arterial or urologic disease.

Our previous observations indicated that both kidneys are affected equally, but since conventional clearance methods may not reveal the significant differences we have integrated these with sodium, solute and water excretion.

Incidence of functional disparity was determined in 50 patients with essential hypertension with minimal if any renal impairment, and in 21 normotensives.

In the normotensive group, 90 per cent showed differences less than 15 per cent of R-L/mean, a limit arbitrarily taken as "normal." In 13 of 50 hypertensives function was "equal" on the two sides.

Thirty-seven of the 50 hypertensives, however, exceeded this limit. This group showed two patterns of impairment. In 20 GFR, C_{PAH}, Tm_{PAH}, $U_{Na}V$, $U_{osm}V$ and V showed disparity, with equal Na and solute excretion fractions. This pattern suggests disparate loss of nephrons. In 17, only $U_{Na}V$, $U_{osm}V$, V and the Na and solute excretion fractions were disparate. This pattern suggests excessive Na and water reabsorption or diminished filtered load in one kidney.

Only two patients of the 37 had low V and U_{Na} unilaterally. One had hyperaldosteronism with tubular vacuolization; the other had focal tubular atrophy and hypertension not relieved by nephrectomy. Two different lesions, then, may produce identical disparities and this particular disparity does not always identify a kidney responsible for hypertension.

It is not established that the two patterns described above are unique or that either is causally related to the pathogenesis of essential hypertension. However, frequent occurrence of functional disparities early in hypertension suggests that the kidney may play a role in pathogenesis.

Serum Properdin and Complement Levels in Normal Dogs and in Dogs with Staphylococcal Bacteremia. A. L. BALTCH, W. OSBORNE, P. BUNN,* L. CANARILE and A. HASSIRDJIAN, Syracuse, N. Y.

Serum properdin and complement titers were studied in dogs to determine concentrations and variabilities, effects of an arteriovenous shunt and effects of a predictable bacteremic infection with and without such surgery. Results were compared with titers found in normal humans and spontaneous human infections.

In 15 normal dogs properdin titers averaged 23 PhN_{50} per ml. (50 per cent bacteriophage neutralization end-point). Complement titers averaged 25 $C'H_{50}$ per ml. (50 per cent hemolytic end-point). Weekly determinations of both for 12 to 20 months revealed each varying within a range of 5 to 20 per cent, uncommonly as much as 50 per cent.

Aorta-inferior vena caval shunts were produced in 16 dogs; eight survived. Stress did not alter presurgical titers of properdin or complement.

Five operated and seven normal dogs were injected

intravenously with 60 million staphylococci (coagulase positive, mannitol fermenting) for seven consecutive days. Resulting bacteremia persisted nine to 28 days. Six of the 12 died—three operated ones (two had endocarditis) and three nonoperated. Two other operated animals developed staphylococcal disease spontaneously; both died with endocarditis. During the course of these eight fatal infections, properdin and complement titers declined gradually.

In surviving dogs, properdin and complement titers did not change six, 12 and 24 hours after first injection. Maximal titer (above normal for each animal) was attained between the third and tenth days. Gradual decline to normal levels occurred during the next seven to 20 days.

Properdin titers in normal humans were lower than in dogs; complement levels were similar. With infection, titers in dogs and man follow similar patterns, *i.e.*, declining properdin and complement titers occur in patients who, unable to overcome infection, succumb, while rising titers develop in those who overcome infection and survive.

Factors Influencing the Renal Excretion of Magnesium in Man. EARL S. BARKER and JOHN K. CLARK,* Philadelphia, Pa.

By acute clearance techniques and various experimental procedures, an attempt was made to determine at least the correlates of magnesium excretion. In 70 studies in normal humans, procedures included intravenous infusions of sodium lactate, sodium acetate, sodium bicarbonate, sodium chloride, acetazolamide (Diamox®), probenecid (Benemid®), *p*-aminohippurate (PAH) (to tubular saturation), magnesium salts, calcium salts and mercurial diuretics. We previously reported temporary increase in urinary magnesium excretion rate (UV_{Mg}) to nearly 300 per cent of control on infusion of hypertonic lactate. Except for acetazolamide, every other procedure caused a significant increase in UV_{Mg}, although that following probenecid was quite small. Following acetazolamide UV_{Mg} decreased somewhat (less than 20 per cent of control) in nine out of 10 studies, possibly related to a fall in glomerular filtration rate (about 15 per cent of control). Absence of demonstrated *increases* in reabsorbed Mg in these human studies is consistent with our previous suggestion, based on magnesium loading in dogs, that possibly tubular transport processes normally operate at or near saturation. If tubular secretion of Mg exists, we have not encountered circumstances where it clearly exceeds simultaneous reabsorption (*i.e.*, "net" secretion).

The excretory pattern for magnesium was distinctly different from that of simultaneously observed excretion rates for Na, K, Cl and PO_4. Changes in UV_{Mg} were usually quite similar to those in calcium excretion, although somewhat smaller in both per cent and in absolute units. Following acetazolamide, however, UV_{Mg} went down while excretory rates for calcium and other ions increased. Apparently renal mechanisms regulating magnesium excretion are, at least partly, separate from those of other ions or the usual acid base factors.

Increase of Aldosterone Secretion by Carotid Artery Constriction and its Prevention by Thyro-Carotid Arterial Junction Denervation. FREDERIC C. BARTTER,* IVOR H. MILLS and DONALD S. GANN, Bethesda, Md.

Whereas the vagus has been shown to mediate stimuli decreasing aldosterone secretion, no comparable neural pathway has been described mediating stimuli for its increase. In the present experiments, adrenal venous aldosterone was measured in dogs by the physico-chemical method of Mills, and brachial and lingual arterial, femoral venous and right atrial pressures were recorded. Low, bilateral common carotid constriction consistently increased aldosterone secretion within 60 minutes, as systemic pressure rose. The increase did not occur if the carotid arteries had been stripped of nerves to above the bifurcation one to two weeks previously; reflex hypertension was also abolished. Denervation of the carotid sinus alone, however, virtually abolished reflex hypertension without affecting the aldosterone response. In contrast, denervation of the thyroid-carotid arterial junction alone abolished the aldosterone response, leaving the reflex hypertensive response intact.

Supradiaphragmatic inferior vena cava constriction reproducibly increases aldosterone secretion in the dog. Thyroid-carotid denervation abolished the increase from this stimulus as well.

The results suggest a dual control of aldosterone secretion, wherein stimuli mediating decreases follow vagal pathways, whereas the major pathway mediating increases derives from the area of the thyroid-carotid arterial junction. They provide a mechanism whereby a localized decrease of intra-arterial blood volume can increase aldosterone secretion independently of total blood volume.

Effects of Glucose Infusions on Serum Lipids and Lipoproteins in Nephrosis. JAMES H. BAXTER,* HOWARD C. GOODMAN and ELEAZAR SHAFRIR, Bethesda, Md.

Serum lipids were studied in 14 subjects during and following continuous infusions of 4 L. of 10 per cent glucose over a 48 hour period. Regular diet was continued. In 10 cases (five controls, five of nephrotic syndrome) there was a decrease or little change in serum triglycerides. Cholesterol decreased in nine of these cases (including all controls) and increased in one; the minimum levels (averaging 86 per cent of the original) occurred at the end of, or 24 hours after, the glucose infusions. In a single case of familial hypercholesterolemia, triglycerides increased moderately and cholesterol decreased.

In the remaining three cases (of nephrotic syndrome) there was a large increase in very low density ($D < 1.019$) lipoproteins, and in serum triglycerides and lactescence. Triglycerides increased by 300 to 850 mg. per cent. This result was obtained five times in one

case. Although a greater decrease in the D 1.019 to 1.063 lipop oteins occurred in these three cases than in the others, serum cholesterol did not change greatly because the decrease in cholesterol of the D 1.019 to 1.063 fraction was counterbalanced by an increase in cholesterol in the D < 1.019 fraction. Similar but greater lipid alterations occurred with oral administration of 1,500 Gm. of glucose in 48 hours. Isocaloric substitution of infused glucose for other food (in one case) produced less effect. Results of administration of glucose-C^{14} while triglycerides were increasing in one case suggested that the infused glucose contributed to the production of the triglycerides. Unesterified fatty acids were depressed in all cases during glucose administration. The changes in lipoprotein patterns induced by the glucose administration, and particularly the differences in the changes which occurred among apparently similar patients with nephrosis, are of interest in relation to the greatly different lipoprotein patterns which we have previously observed among patients with nephrosis.

Insulin-Like Activity of Human Serum Fractions Obtained by Preparative Electrophoresis. PAUL M. BEIGELMAN, Los Angeles, Cal. (Introduced by Paul Starr).

Pooled, normal human serum was fractionated by the technique of preparative electrophoresis, employing the Spinco continuous flow electrophoresis apparatus. Discrete serum protein fractions, characterized electrophoretically by analytic electrophoresis (Durrum method), were concentrated by dialysis against polyvinyl-pyrrolidone. The concentrated fractions were then diluted, usually one to 10, in Krebs-bicarbonate buffer containing glucose and insulin-like activity determined by a method of assay utilizing glucose uptake by Wistar rat adipose tissue. This method has demonstrated sensitivity to as little as 10 μU of insulin per ml.

Fractions tested included albumin, alpha-2-globulin, beta-globulin, gamma-globulin and two other discrete fractions having mobilities between alpha-2- and beta-globulin and between beta- and gamma-globulin. Nonprotein material obtained in the course of preparative electrophoresis was also diluted in Krebs-bicarbonate-glucose and these uptake values constitute the baseline control. Insulin-like activity of the control and various fractions, expressed as mg. glucose uptake per Gm. adipose tissue, are as follows (geometric mean): control, 2.75; commercial serum albumin control, 2.92; albumin, 3.02; alpha-2-globulin, 2.70; fraction with mobility between alpha-2- and beta-globulin, 3.84; beta-globulin, 4.72; fraction with mobility between beta- and gamma-globulin, 4.23; gamma-globulin, 2.42. The maximum insulin-like activity is associated with beta-globulin and is highly significant. The adjacent beta-gamma-globulin fraction also demonstrates significant insulin-like activity. There is equivocal insulin-like activity in the alpha-2-beta-globulin fraction. No insulin-like activity was evident in the albumin, alpha-2-globulin or gamma-globulin fractions.

The Effect of Desoxycorticosterone on the Unidirectional Transfers of Sodium and Potassium into and out of the Dog Intestine. EUGENE Y. BERGER, New York, N. Y. (Introduced by John H. Laragh).

Chronic closed-end isolated sections of bowel 20 cm. in length were prepared in the dog. With the use of Na^{24} and K^{42}, and methyl cellulose as a volume indicator, simultaneous measurements of the unidirectional fluxes of sodium and potassium were made. When the control experiment was completed, desoxycorticosterone acetate (DCA) was administered intramuscularly (2 mg. per Kg.) and measurements were repeated 20 hours later.

The administration of DCA did not alter the transfers of sodium or potassium across the small intestine in studies on two dogs, one with a jejunal pouch, the other with an ileal pouch.

In studies on four dogs with pouches fashioned from the large intestine, DCA decreased the amount of sodium and increased the amount of potassium in the colon lumen. The mean sodium flux into the colon lumen was 28.0 ± 2.0 μEq. per minute in the control, which did not differ from 26.6 ± 1.7 μEq. per minute after DCA. The mean sodium flux out of the lumen was 55.2 ± 2.5 μEq. per minute which increased to 66.7 ± 3.3 μEq. per minute after DCA. The net decrease in sodium in the colon lumen after DCA is consequently the result of an increased flux of sodium out of the lumen. Potassium transfers into and out of the colonic lumen are both increased by DCA. Potassium transfer into the colon increased from 3.37 ± 0.52 to 7.17 ± 1.19 μEq. per minute, while transfer out of the colon increased from 2.02 ± 0.32 to 3.14 ± 0.49 μEq. per minute. The potassium entering the colon increased to a greater degree than the potassium leaving the colon, with a net result of an increase in potassium in the colon lumen after DCA. Thus, for the dog colon, these observations demonstrate the manner in which DCA induces a retention of sodium and a loss of potassium.

Serum Protein Patterns in Acute Poststreptococcal Glomerulonephritis. STANLEY H. BERNSTEIN, New Hyde Park, N. Y. (Introduced by Harry A. Feldman).

Conflicting data regarding the changes in serum protein patterns during the course of acute glomerulonephritis may be related to the multiplicity of etiologic agents producing the clinical syndrome.

Twenty-five patients with fairly uniform clinical and laboratory characteristics of acute nephritis were studied. All patients had hematuria, albuminuria, edema and elevated sedimentation rates following untreated acute respiratory illnesses. Throat cultures and serum specimens were obtained the first week of renal disease, three to six months and 12 months later. Antistreptolysin O (ASO), antihyaluronidase (AH), and antistreptokinase (ASK) antibodies and filter paper electrophoretic patterns were carried out on all sera.

Group A beta hemolytic streptococci were isolated on

culture from 14 patients and from family contacts of two other patients. Elevations of ASO titers were demonstrated during the acute renal disease in 24 patients. Prolonged elevations of ASO were observed in 50 per cent of these patients. AH and ASK antibodies were not consistently elevated. One patient, without demonstrable antibodies, had a Type 12 streptococcus isolated. Serum albumin was consistently decreased during the acute illness, varying inversely with the degree of albuminuria and edema. Alpha 2 globulin was elevated and related inversely to serum albumin levels. Both fractions returned to normal with clinical improvement. Gamma globulin levels were elevated in 10 patients during the acute disease and in four sera three to six months later. Significant correlation between gamma globulin levels and ASO titers was apparent (at the 5 per cent level). Beta globulin and alpha 1 fractions were not consistently affected.

The data suggest that serum albumin and alpha 2 globulin changes, associated with acute poststreptococcal glomerulonephritis, are related to the clinical severity of the disease. The changes in gamma globulin, however, appear to be specifically related to the immunologic response of the host to the previous streptococcal infection. Since the gamma globulin elevations reflect antigenic stimulation, this may be the explanation for the elevated levels of this protein fraction previously reported in this disease.

Large Dose Chemotherapy of Advanced Neoplastic Diseases Preceded by Hematopoietic Stimulation. H. R. Bierman,* K. H. Kelly, G. J. Marshall and R. L. Byron, Jr., Duarte, Cal.

One of the major factors limiting anti-neoplastic chemotherapy is the accompanying hematodepression. Severe prolonged hematodepression is usually attained long before the maximum anti-neoplastic effect despite the rearrangement of dose schedules, AET, cysteine, frequent blood transfusions and other protective maneuvers.

The hematodepressant effects of therapeutic doses of chemical agents have been related inversely to the size of the active bone marrow compartment and the leukocyte reservoirs. Consequently, attempts were made to increase the size and activity of productive hematopoiesis.

Nine patients with advanced metastatic neoplastic diseases other than leukemias or lymphomas underwent hematopoietic stimulation by leukocyte withdrawal (leukapheresis) of 19 to 150 billion leukocytes during each procedure. No untoward effects during any of the 15 leukaphereses were observed.

The amine mustard (HN_2) was employed because of its well-documented behavior under maximal conventional conditions. Maximal hematopoietic stimulation was reached at 48 to 72 hours at which time 1 to 4.2 mg. per Kg. per body weight of HN_2 was administered. Profound but transient hematodepression was observed as anticipated but prompt recovery from the hematodepression began within 10 to 18 days after administration of the drug. In no case was prolonged hypoplasia observed.

Regression of metastatic lesions was documented in four instances, which, although temporary, were remarkable.

The quantitative and beneficial effects of marrow stimulation associated with anti-neoplastic chemotherapy appear to be mediated through the elaboration of a circulating hematopoietic stimulant.

The Influence of Protein Binding on I^{131}-Diodrast Excretion. Jerome B. Block, Dorothy E. Graham and Belton A. Burrows,* Boston, Mass.

We have found that the renal clearance of I^{131}-diodrast in normal subjects is reduced at plasma concentrations below 1 μg. per cent. Equilibrium dialysis studies and simultaneous renal clearances of I^{131}-diodrast and stable diodrast have been employed to evaluate the influence of protein binding of I^{131}-diodrast on its renal excretion.

In vitro, some serum protein binding of I^{131}-diodrast occurred at all concentrations below 1 mg. per cent. At concentrations below 1 to 2 μg. per cent, a relatively nondialyzable complex of I^{131}-diodrast with serum proteins was demonstrated. Added stable diodrast displaced only a fraction of this nondialyzable material.

Renal clearance of I^{131}-diodrast at plasma concentrations in the range of this increased protein binding (below 1 μg. per cent) was less than 350 ml. per minute. A subsequent infusion of stable diodrast, which was cleared at 650 ml. per minute, did not increase I^{131}-diodrast clearance. However, administration of both a prime and sustaining infusion of stable diodrast along with I^{131}-diodrast resulted in normal simultaneous clearances of both compounds at plasma concentrations of I^{131}-diodrast below 1 μg. per cent.

These studies suggest that the complex of plasma protein and I^{131}-diodrast is less dissociable at concentrations below 1 to 2 μg. per cent, becoming a limiting factor in the renal excretion of I^{131}-diodrast. Sites of this increased binding, at different loci on the same plasma protein or on different plasma proteins, may be saturated by stable diodrast; the clearance of I^{131}-diodrast is then independent of plasma concentrations at low levels.

Polymorphism in Thyroxine-Binding Serum Proteins of Man and Other Mammals. B. S. Blumberg and J. Robbins,* Bethesda, Md.

Serum protein binding of I^{131}-labeled L-thyroxine was studied using starch gel electrophoresis [borate buffer, pH 8.6 (Smithies)] and autoradiography.

In humans, four distinct radiothyroxine bands were seen: a fast moving band coincident with the first prealbumin zone, a second band at the leading edge of the albumin zone, a third diffuse band in the trailing portion of albumin, and a fourth band just behind albumin. At approximately 1 μg. per ml. of added thyroxine, the fastest band contained more radiothyroxine than did the other bands. At approximately 0.1 μg. per ml., relatively more

radioactivity was in the fourth band which, therefore, corresponds to alpha-globulin bound thyroxine as seen in paper electrophoresis (barbital buffer, pH 8.6).

Radiothyroxine patterns were qualitatively identical in American whites and Negroes, Alaskan Eskimos and Micronesians, and there were no differences between sera representative of the three major haptoglobin phenotypes. In Alaskan Indians, however, there was a fifth slower-moving radioactive band in the postalbumin region.

In the rhesus monkey (*Macaca mulatta*), a polymorphism in the prealbumin proteins has been noted (Blumberg and Gentile). The fastest radiothyroxine band revealed a similar polymorphism coinciding with each of the three prealbumin types. The other three bands were similar to those in man.

Three other mammals were studied: Guernsey cow, Alaskan fur seal (*Callorhinus ursinus*) and Arctic marmot (*Marmot caligata broweri*). The prealbumin radiothyroxine band was absent in all, and the second band was also absent in the seal and marmot. The cow had an additional prominent band slower than the others.

The possibility that the variations in humans and monkeys are genetically determined is under investigation.

The Role of the Autonomic Nervous System in Human Lipid Metabolism. MORTON D. BOGDONOFF, ARNOLD M. WEISSLER, FRANCIS L. MERRITT, JR., WILLIAM R. HARLAN and E. HARVEY ESTES, JR., Durham, N. C. (Introduced by Eugene A. Stead, Jr.).

Preliminary studies in this laboratory have demonstrated marked elevations in serum nonesterified fatty acid (NEFA) levels during acute emotional arousal. Comparable NEFA rises occur during epinephrine and norepinephrine infusion. These observations, suggesting the presence of a neurohumeral mechanism in NEFA metabolism, have prompted the present investigation of acute emotional arousal, with and without ganglionic blockade, upon serum NEFA in normal subjects.

Serial venous serum NEFA levels were obtained in fasting male subjects observed during a variety of situations: at prolonged rest; during discussion of emotionally-charged life experiences; during a student oral examination; and during a painful, restricting experience. Independent evaluation of emotional arousal was made by observation of the subject during the stressful experience and later by a focused interview. Pulse and respiratory rates and arterial blood pressure were monitored.

Emotional arousal was accompanied by marked increases in serum NEFA levels (+ 50 to 2,000 μEq. per L., representing a 10 to 250 per cent increase over baseline values) within 15 to 30 minutes after initiation of the stimulus. All subjects reported an affect of anxiety during the stimulus; two noted in addition feelings of "hostility." Pulse and respiratory rate increases occurred in all; blood pressure rises were less consistent. During the infusion of trimethaphan camphor sulfonate (Arfonad,® a rapidly metabolized ganglionic blocking agent), resting NEFA values were markedly reduced (− 50 to 150 μEq. per L.), and the NEFA response to arousal was inhibited.

The rise of the serum NEFA levels following "stressful" experiences, the inhibition of this response by the ganglionic blocking agent, and the known rise in the NEFA levels that occurs after epinephrine and norepinephrine infusion suggest an important relationship between autonomic nervous system activity and lipid metabolism in the human.

Extraocular and Neck Muscle, and Thyroid Gland Mucopolysaccharide Response to Thyroid Stimulating Hormone. ALFRED J. BOLLET, RALPH F. KNOPF and WILLIAM H. BEIERWALTES, Detroit and Ann Arbor, Mich. (Introduced by Gordon B. Myers).

Exophthalmos and pretibial myxedema seen in some patients with thyrotoxicosis have been attributed to a direct effect of thyroid stimulating hormone (TSH). We have found a generalized increase in mucopolysaccharide concentration in the skin of patients with pretibial myxedema. In the present study the effect of exogenous TSH on the concentration of acid mucopolysaccharide in the thyroid gland, eye muscle and neck muscle of the dog was measured.

One lobe of the thyroid gland and the extraocular muscles of one eye of each of five dogs were removed and studied histologically and chemically. Neck muscle was similarly studied in four of these dogs. The contralateral lobe of the thyroid gland, extraocular muscles and a neck muscle were removed after the daily administration of 10 units of TSH subcutaneously for three to 13 days. Two untreated dogs served as controls. Using carbazole and orcinol methods of determination of uronic acid, an increase in the concentration of acid mucopolysaccharide in each of these tissues occurred on TSH treatment. The increases in the mean values were: 78 per cent for thyroid gland, 114 per cent for extraocular muscle, and 175 per cent for strap muscle. The thyroid lobes removed after TSH treatment showed hypertrophy, hyperplasia and loss of colloid. Extraocular and neck muscle increased in mass and showed histological changes consisting of swelling of muscle fibers, increased longitudinal striations, loss of cross striations, vacuolar change and variability of staining. Tissue from the two dogs not given TSH showed no increase in acid mucopolysaccharide concentration or alteration in mass or histology.

These findings add evidence to the concept that TSH causes a widespread increase in the concentration of acid mucopolysaccharide.

Separation and Characterization of Aging Red Cell Populations. JOSEPH R. BOVE, Boston, Mass. (Introduced by Charles Phillips Emerson).

Group-O erythrocytes have been isolated from mixtures containing a preponderance of Group-A or Group-B cells by a technique of selective agglutination which permits the recovery of 40 to 80 per cent of the "O" population. Utilizing this technique suspensions composed of 80 to 90 per cent "O" cells have been prepared from samples in which these cells represented no more than 5 to 10 per

cent of the mixture. Osmotic hemolysis tests on incubated and unincubated samples established that no change in erythrocyte osmotic fragility resulted from this separation.

A series of normal Group-A individuals received 250 to 300 ml. of Group-O cells labeled with chromium^{51}. Venous samples were collected at intervals, and the donor cell population was studied for osmotic activity and chromium^{51} content. The osmotic fragility of cells which had circulated in the recipient as long as 72 days was practically unchanged from that of the original donor blood. It was demonstrated that the specific chromium^{51} activity in the erythrocytes had declined exponentially since transfusion. The half-time of disappearance was approximately 70 days. This finding is in close agreement with the data obtained earlier by indirect methods.

These data suggest that: *1)* Chromium^{51} is "eluted" *in vivo* at a predictable rate from intact erythrocytes; and *2)* that the osmotic fragility of donor cells 60 days after transfusion (60 to 120 days old) is normal.

Catalase Activity of Infected Urine. ABRAHAM I. BRAUDE,* Pittsburgh, Pa.

Catalase occurs in nearly all bacteria that produce infection of the urinary tract. Catalase is also present in inflammatory cells and renal tissues. Catalase from these three sources would be expected, therefore, to appear in the urine during infections of the urinary tract. The following study was undertaken to determine whether detection of urinary catalase could be used as a simple bedside test for rapid identification of urinary infection.

Catalase was detected by mixing equal parts of urine and 3 per cent H_2O_2. Catalase rapidly decomposed the H_2O_2, and bubbles of oxygen rose to the surface. The urines of 23 normal men showed no catalase activity; the urines of two other normal men produced only a few tiny bubbles in one hour. The urines of 60 hospitalized patients, collected with strict aseptic precautions, were examined for catalase activity, cultured by quantitative methods, and the sediment examined for erythrocytes, leukocytes and bacteria. Thirty-eight of these urines contained more than 10,000 bacteria per ml., and all 38 possessed catalase activity ranging from moderate to explosive. One urine, containing 3,000 colonies *Proteus mirabilis* per ml., exhibited no catalase activity. The remaining 21 urines were sterile, or nearly so, and eight gave positive catalase reactions. Two of these eight urines were bloody, and three were from patients who had recently received antibiotics for urinary infections. A strongly positive reaction occurred in sterile urine from a patient with renal actinomycosis, identified after nephrectomy. Two positives also occurred with sterile urines of a severe diabetic, and a leukemic with leukemic cells in the urine.

These results suggest that catalase appears in the urine only in diseases of the urinary tract, that the usual cause of catalase in urine is infection, and that the catalase test is a simple, rapid method for bedside detection of urinary infection.

Injections of Radioactive Krypton (Kr^{85}) Solutions in the Detection and Localization of Cardiac Shunts. EUGENE BRAUNWALD, ROBERT T. L. LONG and ANDREW G. MORROW, Bethesda, Md. (Introduced by Robert W. Berliner).

Following injection of krypton in solution into a systemic vein or the right side of the heart, approximately 95 per cent is cleared during one passage through the pulmonary circulation. Arterial blood activity is low when radioactive krypton (Kr^{85}) is injected in the absence of, or distal to, the origin of a right-to-left shunt. However, when injected proximal to the origin of such a shunt, a fraction of the Kr^{85} by-passes the pulmonary capillary bed and appears in arterial blood. Thirty to 50 μc. Kr^{85} was injected through a catheter into the right side of the heart and arterial blood was sampled continuously during the next 15 seconds. In 17 patients proved not to have right-to-left shunts, the activity per ml. of arterial blood was always less than 1.13×10^{-6} and averaged $4.4 \pm 4.3 \times 10^{-8}$ of the total radioactivity injected. In five patients, subsequently shown to have small right-to-left shunts, the radioactivity per ml. of arterial blood was greater. It always exceeded 1.42×10^{-5} and averaged $4.0 \pm 2.6 \times 10^{-5}$ of the total activity injected. Following injection into the left side of the heart proximal to the origin of a left-to-right shunt, Kr^{85} promptly arrives in the pulmonary vascular bed and immediately appears in the expired gas where it may be readily detected. Following such injections into 17 patients, Kr^{85} appeared in the expired gas in an average of 3.7 ± 2.1 seconds and its concentration increased abruptly. However, after injection distal to the origin of a left-to-right shunt of 12 patients, the appearance of Kr^{85} in the expired gas was delayed to a mean of 16.6 ± 6.9 seconds by its slow traversal of the systemic circulation and the expired gas concentration rose gradually. The techniques described are simple to apply and sufficiently sensitive to detect and localize even small cardiac shunts.

An Analysis of the Intrarenal Mechanisms for Sodium Excretion in the Diseased Kidney Employing an Experimental Model with One Normal and One Diseased Kidney. NEAL S. BRICKER,* PETER A. F. MORRIN, S. WESLEY KIME, JR., RAYMOND G. SCHULTZE and CLAIRE KLEIN, St. Louis, Mo.

The ability of the patient with advanced chronic Bright's disease to maintain Na balance is a striking and incompletely understood phenomenon. In the present studies, Na excretion by chronically diseased kidneys was examined and compared with simultaneous functions of normal control kidneys in dogs with *unilateral* renal disease. Three types of severe unilateral disease were induced: *1)* aminonucleoside-nephritis; *2)* pyelonephritis; and *3)* glomerulonephritis. Involved kidneys typically became contracted and demonstrated marked anatomic abnormalities of persisting nephrons.

The ability of the diseased kidney (D.K.) both to conserve Na and to increase sodium excretion in a manner

comparable to control kidneys was documented repeatedly. During stop-flow experiments, D.K. was capable of elaborating Na-free urine (in distal tubular samples) despite high concentrations of mannitol. During water diuresis, D.K. typically reabsorbed > 99 per cent of filtered Na. On a Na-free diet, D.K. excreted < 2 mEq. Na. per 24 hours. When BUN was chronically elevated (exogenous urea administration), the capacity to conserve Na on a Na-free diet persisted.

During 5 per cent NaCl infusion, mercurial diuresis and osmotic diuresis (mannitol, urea, glucose or *p*-aminohippurate infusion), natriuresis by diseased kidneys paralleled that by the contralateral normal organs.

In most experiments, D.K. excreted a greater percentage of filtered Na than normal kidneys. That this was not due to abnormalities in persisting nephrons is suggested by an identical pattern in the dog with a unilateral hemi-infarcted kidney in which persisting nephrons are uninvolved by progressive renal disease. When GFR of D.K. was decreased slightly during mannitol diuresis by partially constricting its renal artery, the difference between the two kidneys disappeared.

It is concluded that persisting nephrons of diseased kidneys *retain essentially normal Na transport mechanisms.* In chronic bilateral renal disease, continuing ability to excrete a large fraction of filtered Na and limited ability to elaborate Na-free urine may relate to an adaptive increase in GFR per nephron rather than to intrinsic abnormalities of persisting nephrons.

Synthesis of Precordial Leads: A Clinical Study of the Dipole Hypothesis of Electrocardiography. STANLEY A. BRILLER and ROBERT H. OKADA, Philadelphia, Pa. (Introduced by Francis C. Wood).

The physical basis of vectorcardiography and of the spatial ventricular gradient is dependent upon the dipole hypothesis. Frank's torso model studies indicated near-perfect agreement with dipole theory in one normal subject. Corroborative evidence provided by cancellation and mirror image studies has been challenged since the results were expressed in terms of unavoidably biased coefficients.

Present studies are based upon a principle outlined by Becking: A consequence of dipole theory is that any body surface voltage must be capable of synthesis by linear combination of three other independent surface voltages. An electronic computer was constructed with which synthesis of multiple precordial voltages (usually V_1 through V_5) could be attempted by summing adjustable proportions of voltages at the right arm, left arm and left leg. Each was measured with reference to a point on the back. The synthesized precordial voltage was electronically subtracted from the actual precordial voltage. The difference so obtained, expressed as a percentage of the actual precordial voltage, gave an index of the degree to which the dipole hypothesis failed to account for the observed precordial voltage.

In 23 normal subjects the average index for each of five precordial locations was: V_1, 13 ± 8 per cent; V_2, 16 ± 11 per cent; V_3, 15 ± 7 per cent; V_4, 19 ± 11 per cent; V_5, 13 ± 8 per cent. The smallest indices in this group (V_1, 6 per cent; V_2, 4 per cent; V_3, 10 per cent; V_4, 11 per cent; V_5, 6 per cent) were obtained from the subject utilized by Frank in torso model studies. In 28 subjects with heart disease of various etiologies, values for the same precordial sites were: V_1, 35 ± 30 per cent; V_2, 38 ± 26 per cent; V_3, 38 ± 14 per cent; V_4, 31 ± 26 per cent; V_5, 28 ± 23 per cent. These data indicate that in the presence of diseased (and occasionally healthy) myocardium, sizable voltages appearing on precordial electrodes frequently can neither be detected nor analyzed by methods such as vectorcardiography.

The Nature of Urinary Acidification Process During Transient Obstruction of Renal Artery and Ureter. WILLIAM A. BRODSKY,* JOHN T. KAIM and GASPAR CARRASQUER, Louisville, Ky.

Nembutalized dogs were subjected to loading with mannitol, or with mannitol plus Na_2SO_4, $NaHCO_3$ or Ringers solution. After steady-state levels of urinary pH and flow had been reached, the renal artery was clamped for one to three minutes. After release of the clamp, urine was collected in 1 to 2 ml. aliquots at an excretion rate of 10 ml. per minute per kidney. Similar experiments on ureteral clamping (10 to 20 minutes' duration) were performed. Urine pH decreased by as much as 1.5 units in the first minute after clamp release, and then returned to control levels, in both types of experiments.

Transient changes of urinary pH, Na and K after release of artery clamping were closely similar to those found after release of ureteral clamping. Potentiometric titration revealed the appearance of a weak organic anion of pK 4.8 in postclamping samples of urine. The buffering action was not present in preclamping samples, and urinary creatinine accounted for no more than 10 per cent of the effect. Hydrostatic pressure in the renal pelvis during ureteral clamping was two-thirds of the concurrently determined carotid arterial pressure, while renal blood flow, measured rotametrically, decreased by one-third. The similarity of urinary ionic composition and buffering activity after both arterial and ureteral stop-flow, and the compression of intra-pelvic kidney tissue during ureteral clamping suggest the following: *1*) an interference with the flow of blood through the renal papillae during ureteral clamping; and *2*) the post-clamping increase of urine acidification is due, in part, to continuing distal function, and in part, to anoxia of renal papillary tissue.

A Unified Concept of the Electrocardiographic Image Torso. DANIEL A. BRODY, Memphis, Tenn. (Introduced by James W. Culbertson).

The electrocardiographic image torso is a point-by-point transformation of the body surface, serving as a nomogram of the heart-lead relationships of all leads derived from surface electrodes. Characteristic of this

function, a line joining two points of the image torso represents the lead vector of the corresponding body surface lead. Clinical inaccuracies of the image torso concept depend largely upon deviations of the heart from single, fixed-location dipole behavior.

Image torsos of variously shaped volume-conductors studied by others all bear a striking configurational resemblance to each other. This suggests that one or more fundamental principles, largely independent of body configuration, determine the image torso shape. Initial exploration of this possibility in irregularly-shaped, homogeneous, laminar models showed both theoretically and experimentally that their image torsos were exactly circular. Stereoscopic image torso views derived from a rectangular volume-conductor revealed a relatively simple, gently rounded configuration, which was not greatly altered by significant distortion of the volume-conductor shape. Finally, a three-dimensional model of the Frank image torso, constructed of white beads mounted on black threads and photographed while rotating, closely approximated an oblate spheroid except for small but significant bulging in the left shoulder region.

On the basis of principles governing dipolar current flow in extended media, and from consideration of boundary conditions imposed by limiting surfaces, we can now explain qualitatively the relative independence of image torso configuration from volume-conductor shape. Paradoxically, the image torso concept is probably limited in ordinary clinical utility because, if cardiac behavior is essentially dipolar, relatively few leads are required to provide all available diagnostic information. Contrariwise, the unified concept developed here offers a powerful investigative means of determining whether the heart really behaves as a single, fixed-location, current dipole.

Candida Reacting Antibody in the Serum of Patients with Lymphomas and Related Disorders. JEROME I. BRODY and STUART C. FINCH,* New Haven, Conn.

The purpose of this study was to evaluate the effects of neoplastic growth and tumor therapy on serum antibody capable of reacting with the yeast form of *Candida albicans* and to determine its importance in the pathogenesis of Candida infections. Serum antibody reactions to Candida and rice starch antigen in patients without lymphomas and in patients with cancer, leukemia and lymphomas were investigated by the technique of immune-adherence.

Immune-adherence involves the attachment of antigen to the surface of primate erythrocytes only in the presence of antibody and complement. The adherence of particulate antigen to the red cell may be visualized microscopically or may be indicated macroscopically by red cell floccule formation. This assay provides certain advantages over conventional immunological tests in that in general it is about five times as sensitive as complement fixation and is extremely simple to perform with a high degree of precision. The results of these studies indicate that serum Candida reacting antibody and starch antibody are acquired with aging and apparently do not represent inherited serum reacting antibody. In general, infants, children and adolescents have low levels of antibody of this type. Adults, however, almost all invariably have high levels, that is, dilutions of serum from 1 to 1,000 to 1 to 4,000 were reactive in inducing immune-adherence.

It appears that antibody reacting with Candida and starch, once fully acquired, remains adequate even when primary antibody production to certain specific proteins becomes depressed. The increased rate of mycotic infections in leukemia and lymphoma patients seems to be independent of serum antibody reactivity and probably is related to other constitutional factors in its pathogenesis.

Detection and Measurement of Experimentally Produced Aortic Regurgitation. LEON BROTMACHER, HIRAM W. MARSHALL, RICHARD J. CHEESMAN and DAVID E. DONALD, Rochester, Minn. (Introduced by Earl H. Wood).

Detection of indicator in the left ventricle immediately after injection into the root of the aorta is proof of retrograde passage across the aortic valve. The proportion of dye regurgitated is related to the ratio, R_1, of the areas of the dilution curves recorded from the left ventricle after injecting equal doses of dye into the root of the aorta and into the pulmonary artery, provided that flow is unchanged during the two injections and that uniform mixing occurs. The proportion of dye regurgitated is also related to the ratio, R_2, of the areas of the curves recorded simultaneously from the left ventricle and femoral artery after an injection into the root of the aorta.

Fourteen dogs were investigated before production of aortic regurgitation via a carotid artery without thoracotomy and 10 were investigated afterward. Dye curves were recorded at the pulmonary artery, left ventricle and femoral artery following injections of tricarbocyanine dye II (cardio-green) into the superior vena cava, pulmonary artery and aortic root.

Aortic and left ventricular pressures were recorded and mean diastolic gradients across the aortic valve were calculated. Retrograde flows of dogs' blood across the aortic valve at varying pressure gradients were measured directly at necropsy. The regurgitation during life was estimated from these data.

No dye was detected in the left ventricle after aortic injections in 10 of 14 control dogs; small amounts ($R_1 =$ 0.02 to 0.04) were noted in four control dogs and much larger amounts ($R_1 = 0.3$ to 1.2) were present in all dogs with aortic regurgitation. There was a positive correlation (0.95 and 0.90, respectively) between R_1 and R_2 and regurgitant flows estimated from perfusion studies.

No or poor correlation was demonstrable between estimated regurgitation and *1*) the disappearance slope or variance of curves recorded at the femoral artery, or *2*) the variation in relationships between simultaneously recorded pulmonary and femoral artery curves.

Control of Fat Metabolism by Growth Hormone in Humans. JOSIAH BROWN and L. R. BENNETT, Los Angeles, Cal. (Introduced by William N. Valentine).

Administration of growth hormone (GH) leads to enhanced catabolism of fat, deposition of new protein and depressed utilization of carbohydrate. The site and mechanism of these effects have not been clearly delineated. This study of the effects of human GH in normal young subjects indicates that enhanced catabolism of fat is an indirect effect resulting from prevention of the inhibition of fatty acid oxidation which normally follows glucose administration.

The rate of oxidation of an intravenous tracer of 1-C^{14} albumin-bound palmitic acid was determined by continuous collection and measurement of all expired $C^{14}O_2$ for one hour. Five normal subjects were studied repeatedly before and four or eight hours after 4 mg. GH (Raben) alone, GH followed by glucose (200 Gm.) or glucose alone on three separate days. Alternatively, studies were performed before and after three doses of GH in 24 hours followed then by glucose and insulin and a final study.

The mean $C^{14}O_2$ outflow in these and eight other normal subjects was 10.4 per cent of administered C^{14} palmitic acid at one hour. GH administration resulted in no change in $C^{14}O_2$ outflow despite increases of two to three times in blood unesterified fatty acids (UFA). Normal subjects' similar response to prolonged fasting is interpreted as a balanced acceleration of mobilization and oxidation of fatty acids. Glucose administration lowered UFA from 0.7 to 0.3 mEq. per L. and reduced $C^{14}O_2$ to one-half the control value. Following GH plasma UFA fell in response to glucose to the same low value but $C^{14}O_2$ outflow was unchanged. Crystalline insulin (10 units I.V. drip in two hours) with glucose did not overcome the inhibited response to glucose.

GH did not increase $C^{14}O_2$ production as seen in hypermetabolic states and chronic undernutrition and did not prevent glucose inhibition of mobilization of UFA but abolished the normal preferential combustion of glucose by peripheral tissues. Within the limits of these methods this appears to be a mechanism for the acceleration of fat oxidation by GH.

Metabolic Studies on Cortisone Sensitivity and Resistance in a Lymphocytic Neoplasm. JOHN H. BRYANT, Bethesda, Md. (Introduced by James A. Shannon).

The availability of cortisone-sensitive transplantable mouse lymphocytic neoplasms from which cortisone resistant sublines have been derived prompted a study of the metabolism of these tumors.

In experiments with the sensitive tumor, progesterone, desoxycorticosterone and testosterone (10^{-6} to 10^{-3} M), incubated *in vitro* with tumor slices, caused a 25 to 75 per cent inhibition of the conversion of C^{14} labeled glucose, galactose and fructose to CO_2; hydrocortisone was less effective. Cortisone was not effective *in vitro* but was inhibitory to carbohydrate metabolism in tumor slices when the tumor-bearing mouse was preinjected with cortisone. Measurement of Q_{O_2} and of $C^{14}O_2$ from variously labeled substrates suggests that the inhibition involves both the hexose monophosphate and glycolytic pathways or some system common to both.

Lineweaver-Burk plots of the data relating inhibition to substrate concentration reveal that the steroid inhibition is of the competitive type. The apparent K_m for the conversion of glucose to CO_2 is about 4×10^{-4} M, whereas the K_I for the testosterone inhibited system is about 8×10^{-6} M, indicating a relatively high affinity of the inhibitor for a component of a rate limiting step.

In experiments with the *resistant* tumor, a similar steroid inhibition of carbohydrate metabolism is found, the magnitude and type of inhibition being the same as in the *sensitive* tumor. However, a significant difference between the two tumors is seen in the rates of glucose utilization. In the absence of steroid, the *resistant* tumor uses one and one-half to three times as much glucose as does the *sensitive* tumor. Glucose utilization by the inhibited *resistant* tumor approximates that of the uninhibited *sensitive* tumor. This is suggested as reflecting a possible mechanism for steroid resistance in this tumor.

Similarity of Serum Protein Changes in Primary and Secondary Amyloidosis. EVAN CALKINS and ALAN S. COHEN, Boston, Mass. (Introduced by Walter Bauer).

Previous studies indicated that amyloid from patients with primary and secondary amyloidosis and rabbits with casein induced amyloidosis appears to be histologically and chemically similar. The present study was to determine whether serum changes previously noted in rabbits are evident in patients with amyloidosis.

Sera were obtained from nine cases of primary amyloidosis (confirmed by biopsy or autopsy), three of whom had nephrosis, and six cases of secondary amyloidosis, similarly confirmed. Control sera were obtained from 20 normals, three nonamyloid nephrotics and patients with chronic inflammatory disease (80 with rheumatoid arthritis and 20 with leprosy). Eight of the nine patients with primary amyloidosis, despite the absence of evident inflammatory disease, exhibited nonspecific serum abnormalities of the sort customarily associated with inflammation. These involved C-reactive protein (positive in six and four plus in three), serum hexosamine concentration exceeding 120 mg. per cent in all and 176 mg. per cent in four, with hexosamine-total protein ratios over 2.7 in five, and electrophoretic changes. Free and paper electrophoresis showed increased α_2 globulins in non-nephrotic and nephrotic sera, although the relative concentration was much higher in the latter. The ninth patient (J.P.) with primary amyloidosis, which was of the familial sort with severe polyneuropathy, exhibited marked elevation in the β_2 fraction, and was the only primary amyloid patient with normal serum hexosamine and negative C-reactive protein. The patients with secondary amyloidosis exhibited electrophoretic patterns, hexosamine concentrations and C-reactive protein levels similar to those with primary disease, excepting J.P.

These data indicate that the majority of patients with primary as well as secondary amyloidosis have significant, though nonspecific, abnormalities in serum proteins, and, together with the similarities in the amyloid itself, provide grounds for considering the possibility of related pathogenetic mechanisms.

The Hypercalciuria of Hyperparathyrodism in Man. JOHN J. CANARY and LAURENCE H. KYLE,* Washington, D. C.

Although conventional concepts attribute rates of urinary calcium excretion to serum calcium concentration, recent investigation suggests that parathyroid hormone increases renal calcium reabsorption, providing an additional mechanism for hypercalcemia. The hypercalcemia and the hypercalciuria of hyperparathyroidism were explored by performance of serial calcium clearances in five hyperparathyroid patients, before and after surgical removal of a parathyroid adenoma, and in a group of 12 normal subjects. Measurement of serum filtrable calcium, glomerular filtration rate and the rate of urinary calcium excretion permitted estimation of the filtered load of calcium, calcium clearance and the rate of tubular reabsorption of calcium.

The mean level and standard deviation of serum filtrable calcium was 5.8 ± 0.12 mg. per 100 ml. in the normal group, whereas the hyperparathyroid subjects had definite elevation of filtrable calcium concentration (mean, 7.4 ± 0.26 mg. per 100 ml.). However, in both groups the per cent filtrable calcium agreed closely (58 to 64 per cent). The mean calcium clearance in normal subjects was 2.6 ± 0.31 ml. per minute and the filtered load of calcium averaged 7.1 ± 0.37 mg. per minute. Calcium clearance was increased in the hyperparathyroid patients before surgery (mean, 3.6 ± 0.6 ml. per minute) and fell to a low normal range postoperatively (mean, 1.9 ± 0.4 ml. per minute). The filtered load of calcium was elevated in the hyperparathyroid subjects (mean, 10.3 ± 0.8 mg. per minute) and fell sharply to within the normal range postoperatively (mean, 7.4 ± 0.54 mg. per minute). The per cent of filtered calcium reabsorbed was normal preoperatively and essentially unchanged after surgery. Tubular reabsorption of calcium in mg. per minute varied directly with the filtered load of calcium before and after surgery.

These data indicate that the renal reabsorption of calcium in man is not directly influenced by endogenous parathyroid hormone, but that the hypercalciuria of hyperparathyroidism results from the increased filtered load of calcium. The effects of parathyroid hormone on human serum calcium levels are apparently extrarenal.

Chemical and Clinical Studies of Bromsulfalein (BSP) Metabolites. JOHN V. CARBONE, G. M. GRODSKY and R. FANSKA, San Francisco, Cal. (Introduced by Maurice Sokolow).

Bromsulfalein (BSP) is present in bile, serum and urine as the free dye and at least two metabolites. This study reports the chemical nature of the metabolites and variations in serum of free and metabolized BSP in patients with liver disease and BSP retention.

The two major metabolites obtained from human bile were degraded with concentrated hydrobromic acid. Three ninhydrin positive moieties were obtained. Their chromatographic mobilities corresponded to glycine, glutamic acid and cysteine. Similar treatment of the bile controls yielded glycine and glutamic acid. Both metabolites gave a positive reaction to potassium dichromate-silver nitrate treatment indicating the presence of unoxidized sulfur. Hydrogenolysis with Raney nickel of both metabolites resulted in a ninhydrin positive moiety whose chromatographic mobility corresponded to alanine (desulfurated cysteine). These studies indicate that the ninhydrin positive nature of BSP in bile is due to its conjugation with cysteine or with cysteine, glycine and glutamic acid.

Free BSP and BSP metabolites were determined in serum 45 minutes after a dose of 5 mg. per Kg. body weight was given. The results were analyzed in 60 patients whose liver function studies supported the clinical diagnosis of viral hepatitis, metabolic cirrhosis, intrahepatic obstruction due to methyltestosterone and extrahepatic biliary tract obstruction. The mean total BSP retentions in viral hepatitis (nine), metabolic cirrhosis (30), intrahepatic obstruction (11) and extrahepatic obstruction (10) were, respectively, 52 per cent, S.E. = 4 per cent; 34.3 per cent, S.E. = 5.5 per cent; 24.5 per cent, S.E. = 5.3 per cent; and 42 per cent, S.E. = 5.4 per cent. The mean BSP metabolite retentions were, respectively, 10.1 per cent, S.E. = 2.6 per cent; 17.7 per cent, S.E. = 1.8 per cent; 40 per cent, S.E. = 2.8 per cent; and 46.5 per cent, S.E. = 6.8 per cent. Only traces of metabolite were found in normal subjects.

Although total BSP retention did not distinguish parenchymal dysfunction from obstruction, the levels of metabolite in the serum were significantly higher in the obstructed cases. This may prove of value in distinguishing these two types of hepatic functional disorder.

Measurement of Mitral Regurgitant Flow: A Modification of the Variance Method Based on Physiologic Principles. RICHARD A. CARLETON, GILBERT E. LEVINSON and WALTER H. ABELMANN,* Boston, Mass.

In previous work with the Korner and Shillingford variance method for quantifying valvular regurgitation, the volume used has been the product of cardiac output (CO) and mean circulation time. With left atrial injections, this "central" volume (CV) has two components: The first, residing in left heart chambers and the single arterial pathway to the sampling site, is the volume in which sampled dye can mix; temporally equivalent arterial pathways, in which sampled dye cannot mix, comprise the second component. Since the variance is affected only by mixing volume, the CV is an overestimate of the desired volume. Therefore, backflows are overestimated. With this method, we have obtained backflows exceeding 500 per cent of forward flow.

The anatomic identity of the residual volume (RV) of Newman is indeterminate, but its physiologic interpretation as a mixing volume is valid. Therefore, its use with the variance method is reasonable.

T-1824 dilution curves from 28 patients without regurgitation were used to derive the equation:

$$\text{Log predicted variance} = 3.1735 - 1.8423 \log \text{CO} + 1.4258 \log \text{RV}.$$

In 44 patients with isolated mitral valve disease, backflows calculated by this modified variance method had a correlation of 0.85 with surgical estimates of severity. Regurgitant flows averaged 0.69 L. per minute (11 per cent of forward flow) in persons without surgically palpable regurgitation, and ranged from 2.6 to 6.9 L. per minute (76 to 164 per cent of forward flow) with essentially pure regurgitation. The standard error of an observation was 6.8 per cent of forward flow.

These results, used with the Gorlins' formula, yielded diastolic valve areas which correlated well with planimetrically measured surgeons' diagrams.

In patients with essentially pure regurgitation, an assumed maximal diastolic valve size was used with the Gorlins' formula to compute backflow estimates. These agreed closely with RV variance method backflows.

The residual volume variance method is valid and provides physiologically reasonable estimates of regurgitant flow.

Inhibitory Effect of Canine and Human Plasma upon Enzymatic Hydrolysis of Steroid Glucuronides. NICHOLAS P. CHRISTY, New York, N. Y. (Introduced by Joseph W. Jailer).

During studies upon rate of steroid glucuronide formation in the dog, it was observed that 17-hydroxycorticosteroid glucuronides could not be found in plasma after infusion of hydrocortisone. Under these conditions, steroid glucuronide was identified in urine. The findings suggested the possibility of a beta-glucuronidase inhibitor in plasma. This interpretation was strengthened by the detection of tetrahydrohydrocortisone glucuronide in plasma following ethanol precipitation of protein.

Inhibitory activity was studied in the following manner. Measured samples of authentic sodium tetrahydrocortisone glucuronidate (sodium salt of 3 alpha, 17 alpha, 21-trihydroxypregnane-11,20-dione monoglucuronide) were incubated at 37° C. for 48 to 96 hours with 2 to 4 ml. canine plasma in the presence of acetate buffer (pH 4.5, 0.1 M) and beef liver beta-glucuronidase (1,500 to 15,000 units). Control enzymatic hydrolysis, using 20 μg. sodium tetrahydrocortisone glucuronidate in water at pH 4.5, released 8.9 to 15.8 μg. tetrahydrocortisone determined by the phenylhydrazine-sulfuric acid reaction (theoretical yield, 12.9 μg.). Using 10 μg., the yield was 5.5 to 7.8 μg. tetrahydrocortisone (theoretical yield, 6.5). With added canine plasma, enzymatic hydrolysis of 20 μg. glucuronidate released 4.4 μg. of tetrahydrocortisone (34 per cent of theoretical yield). Hydrolysis of 10 μg. glucuronidate yielded 1.8 μg. tetrahydrocortisone with 1,500 units enzyme; 2.9 μg. with 15,000 units (28 to 45 per cent of theoretical). Canine plasma did not interfere with colorimetric estimation of tetrahydrocortisone.

Canine urine and cerebrospinal fluid did not inhibit beta-glucuronidase activity. Human plasma inhibited enzyme unless previously extracted with dichloromethane.

It is concluded that canine and human plasma inhibit enzymatic hydrolysis of certain steroid glucuronides. Since inhibition is absent from relatively protein-free body fluids, and is prevented by prior alcohol precipitation, inhibitor may be a protein or protein-bound substance. The precise protein fraction containing inhibitor and the mode of inhibition (*i.e.*, competitive or noncompetitive) are matters under investigation.

Circulatory Effects and Use of Isoproterenol in Differentiating Patients with Pulmonary Venous Hypertension. LEONARD A. COBB, ROBERT A. BRUCE* and JOHN H. MORLEDGE, Seattle, Wash.

Isoproterenol administered intravenously in microgram doses per minute has been reported to decrease "pulmonary capillary" ("PC") pressure, even when the latter is elevated in patients with left ventricular failure (LVF). It also increases heart rate and stroke volume (SV), and decreases systemic and total pulmonary resistances. Further studies have been performed on changes in "central blood volume" (CBV) and in changes in left ventricular filling resistance (mean "PC" × 100)/(mitral valve flow) in patients with LVF and with mitral valve disease. CBV represents volume from pulmonary to brachial arteries, and all temporally equidistant points.

In 15 patients with LVF but no mitral stenosis (MS), the changes in CBV were from −69 to +420 ml. and in SV from −8 to +23 ml. Changes in CBV correlated poorly with changes in cardiac output ($r = +0.37$), but more closely with changes in SV ($r = +0.77$). These data may support the concept that CBV functions as a permissive pool for changes in SV; however, the locus of changes in CBV is not defined.

In seven patients with surgically proven MS, "PC" pressure rose 2 to 21 mm. in response to isoproterenol. Similar responses in five patients with predominant mitral regurgitation (MR), demonstrated at open heart surgery, ranged from −6 to 6 mm., while those in 10 patients with LVF varied from −12 to 1 mm. Left ventricular filling resistance decreased on the average 2.1, 9.1 and 14.1 units in patients with MS, MR and LVF, respectively.

Hemodynamic responses to isoproterenol are valuable in more completely differentiating patients with pulmonary venous hypertension due to mitral valve and left ventricular diseases.

Canine Platelet Lifespan after Freezing with Glycerin-Plasma Solutions. PHIN COHEN and FRANK H. GARDNER,* Boston, Mass.

By the Cr^{51} technique the normal lifespan of the labeled canine platelet is seven to nine days. Using the platelet

lifespan as the major criterion of viability in platelet preservation techniques, the preservation of canine platelets by freezing in glycerin-plasma solutions has been pursued.

Platelet concentrates (P.C.) are prepared by differential centrifugation at 2° C. The concentrate then is tagged directly with 150 μc. $Na_2Cr^{51}O_4$. A solution of 40 per cent glycerin w/v dissolved in platelet-poor plasma is added to the concentrate in order to achieve a final glycerin concentration of 9 per cent w/v. This concentration of glycerin has been found to be optimum within the 5 to 15 per cent w/v range tested. The concentrate is frozen, in a 100 ml. plastic bag or in a 50 ml. siliconed tube, at 1° C. per minute down to minus 15° C. and at 5 to 10° C. per minute down to minus 79° C.

The concentrate is thawed immediately by immersion in a 37° water bath. A solution of 35 per cent dextrose dissolved in platelet-poor plasma is added to the thawed P.C., 3 ml. every two minutes until a total of 12 ml. has been added. The Cr^{51} labeled concentrate is transfused to the donor dog and the platelet lifespan measured.

By this technique the frozen Cr^{51} labeled platelets infused in four animals had a lifespan identical to the seven to nine days obtained by Cr^{51} labeling alone. An immediate destructive survival pattern (one day) results from freezing and thawing P.C. The addition of glycerin to the P.C. prior to freezing yielded a diphasic survival curve in the recipient with the major loss of platelets in the first day. While glycerin offers protection in freezing, the addition of hypertonic dextrose after thawing acts as an osmotic buffer to prevent thrombocytolysis after infusion and allows a normal lifespan of canine platelets.

The Fate of Staphylococci in Suspensions of Polymorphonuclear Leukocytes. ZANVIL A. COHN and STEPHEN I. MORSE, New York, N. Y. (Introduced by James G. Hirsch).

The interaction between staphylococci and rabbit polymorphonuclear leukocytes has been studied *in vitro*. Homogeneous populations of peritoneal exudate granulocytes were suspended in a medium composed of balanced salts, glucose and rabbit serum. The leukocyte concentration was adjusted to 30×10^6 per ml., and washed bacteria added at a 1 to 1 multiplicity. Suspensions were incubated at 37° C. on a reciprocal shaker. At prescribed intervals, aliquots were processed to determine the total number of viable microbial units and the number in the extracellular and leukocyte fractions. From these findings it was possible to delineate quantitatively both the phagocytic and bactericidal activities of the leukocytes.

Strains of avirulent, coagulase negative *S. albus* were rapidly incorporated into and killed by leukocytes in the presence of normal serum. In contrast, virulent, coagulase positive strains of *S. aureus* remained viable under identical conditions. More than 90 per cent of the coagulase positive organisms were still extracellular after three hours. Maintenance of *S. aureus* viability was therefore the consequence of resistance to phagocytosis and not of intracellular survival.

Serum and leukocytes were obtained from rabbits immunized with heat-killed *S. aureus*. Studies of immune serum revealed the following: *1*) Addition of serum prepared against one strain resulted in efficient phagocytosis and intracellular killing of all strains of *S. aureus* tested. *2*) Absorption with *S. albus* and *E. coli* did not alter this capacity. *3*) Absorption with *S. aureus* completely removed opsonins for the absorbing strain, but not for other strains of *aureus* staphylococci. Polymorphonuclear leukocytes obtained from immune rabbits were identical to normal leukocytes in phagocytic and bactericidal properties.

Thus, under these conditions *S. aureus* is: *1*) resistant to phagocytosis; *2*) opsonized by immune serum; and *3*) rendered nonviable once ingested by polymorphonuclear leukocytes.

Demonstration of Circulating Antibody to Ehrlich Ascites Tumor in Mice. LEON R. COLE, RUTH W. HENDERSON and ANN F. WEST, Los Angeles, Cal. (Introduced by Clayton G. Loosli).

Ehrlich ascites tumor was propagated in an inbred strain of white Swiss mice, and these mice developed circulating antibody to the tumor. The tumor was harvested from donors, and extracted with 0.85 per cent sodium chloride. This tumor extract was subjected to an extensive series of fractionation procedures, which consistently yielded an antigen material. The method for isolation of antigen will be outlined in detail. Mouse erythrocytes were treated with tannic acid, and the antigen material was coated onto their surfaces. Sera from mice inoculated with viable tumor 17 to 19 days previously uniformly caused agglutination of these coated erythrocytes. The average serum titer was 1 to 128. Sera from normal control mice uniformly had titers of 1 to 8 or less. In four instances, antigen was prepared from ascites tumor of a single mouse. Serum from the same animal was found in each case to contain an antibody titer of 1 to 64 to its own tumor antigen. Absorptions of sera by antigen-coated erythrocytes caused significant lowering of titers. Inoculations of normal mice with the purified tumor antigen plus adjuvant produced specific antibody response.

This demonstration of antibody to Ehrlich ascites tumor was made possible by a modification of the Boyden tannic acid-treated antigen-coated erythrocyte technique, whereby normal serum was not added to diluent solutions. The method was thereby made more sensitive for detection of antibody, since positive hemagglutination patterns formed more readily in less viscous medium. The present modification was accomplished by the complete removal of trace heavy metals from water used to prepare solutions, employing Eriochrome black T dye as indicator. Erythrocytes had considerably higher tolerance for treatment with tannic acid, when not exposed to traces of heavy metals. They did not require the stabilizing effect of

serum protein to inhibit the formation of nonspecific positive hemagglutination patterns.

The Excretion of Bromsulphalein in Bile as a Conjugate of Glycine and Glutamic Acid. BURTON COMBES, Dallas, Texas. (Introduced by Gladys Fashena).

Although the bromsulphalein (BSP) test is extensively used in the clinical detection of liver disease, the mechanisms by which BSP is taken up by the liver and excreted into the bile remain poorly understood. An analysis of BSP compounds appearing in rat bile and in human T-tube bile after intravenous administration of this dye was therefore undertaken.

Filter paper chromatograms of rat bile revealed four distinct BSP bands, one of which migrated with the same R_f as standard BSP. The mobility of standard BSP was not altered by incubation with bile *in vitro*. The major band, designated BSP-A, accounted for 50 to 80 per cent of the excreted BSP when graded doses (0.25 to 2.65 mg. per 100 Gm. body weight) of this dye were administered. Hydrolysis of BSP-A yielded BSP and two ninhydrin spots on rechromatographing. The latter were identified as glycine and glutamic acid by two-dimensional paper chromatography of the free amino acids and this was confirmed by identifying their dinitrophenyl derivatives. No evidence of a glucuronic acid conjugate was found, and tests for free or combined sulfhydryl groups were negative. When $BS^{35}P$ was administered and radioautographs were made of chromatograms of bile, no colorless BSP compounds were identified.

Usually two and occasionally three BSP bands were observed on chromatograms of human bile. The major compound, which had a different R_f from BSP-A in the rat, is not a glucuronic acid conjugate. Like BSP-A (rat), it contains ninhydrin reacting material which after acid hydrolysis appears to yield glycine and glutamic acid.

These data in man and in the rat indicate that BSP is excreted into bile predominantly as a conjugate of glycine and glutamic acid and suggest, therefore, that the BSP test, at least in part, is a measure of amide formation in the liver.

Observations on the Uricosuric Effects of Zoxazolamine in Gout. THOMAS B. CONNOR, T. NELSON CAREY, THOMAS DAVIS and HARRIS LOVICE, Baltimore, Md. (Introduced by Jacob E. Finesinger).

Zoxazolamine was administered in 1.0 Gm. oral doses daily to 15 gouty subjects in the intercritical or tophaceous stage of the disease. In all subjects the serum uric acid was greater than 7.5 mg. per cent before therapy.

Renal clearance studies were performed in eight of these patients. In each individual there occurred a four- to eightfold increase in urinary urate excretion with the maximum rise demonstrated within 90 to 180 minutes of administration of the initial dose. Serum urate levels declined 1.5 to 3.0 mg. per cent during this same period. There also occurred a four- to tenfold increase in $C_{urate}/C_{creatinine}$ in each subject.

Six subjects were hospitalized and placed on constant diets for 18 to 36 day periods. Following a suitable control period of three to six days, each patient was given zoxazolamine orally in a dose of 0.25 Gm. four times daily. In each instance there was a marked increase in 24 hour urinary urate excretion (always 25 per cent or more above control values) and serum urate concentrations always reached normal levels (< 6.0 mg. per cent) within 24 to 48 hours.

To one subject, who showed a marked uricosuric response to zoxazolamine, aspirin was given for two four day periods (in daily doses of 1.2 and 4.0 Gm., respectively) together with a 1.0 Gm. daily dose of zoxazolamine. In each study period aspirin produced a marked inhibition of uricosuric effect. Another analgesic compound (N-acetyl-*p*-aminophenol) given in a 2.4 Gm. daily dose together with 1.0 Gm. zoxazolamine daily did not alter the sustained uricosuric effect of zoxazolamine.

In four subjects the response to 1.0 Gm. doses of probenecid and zoxazolamine were compared. In three patients, the effect of zoxazolamine on $C_{urate}/C_{creatinine}$ was consistently greater than with probenecid, whereas in the other subject no significant difference could be demonstrated.

One patient with gouty nephritis and azotemia (BUN, 50 to 70 mg. per cent) responded to 1.0 Gm. daily dose zoxazolamine with a twofold increase in urinary urate and decrease in serum urate from 12.5 to 9 mg. per cent over a six day period. Probenecid was given to the same subject, in the same dosage, for a similar period, with no effect on serum and urine urate.

Nine subjects have been maintained on continuous zoxazolamine therapy in 1.0 Gm. daily doses for periods of two to 12 months and in every patient serum urate has remained below 7.0 mg. per cent with sustained maintenance of uricosuric effect, with the exception of one individual who required 1.5 to 2.0 Gm. daily to maintain this state.

Aural tophi completely disappeared in one patient within six months of initiating therapy. Relief of chronic joint pain has been impressive. Side reactions have not been observed in this group.

Evidence of the Recycling of Siderocyte Iron. WILLIAM H. CROSBY,* Washington, D. C.

When erythroblasts accept more iron than they can use for hemoglobin, the excess forms ferruginous granules. Erythrocytes with such inclusion bodies are called siderocytes. The spleen is able to remove these granules from a circulating erythrocyte without destroying the cell itself. Siderocyte iron is recycled and reutilized, moving in the same paths as hemoglobin iron: from marrow to spleen via the red cells, and from spleen to marrow as plasma iron. The concentration of iron in the granules is about 25 per cent; iron in erythrocytes is 0.1 per cent. Therefore, the iron in a 1 μ siderin granule equals that of an entire erythrocyte. One such granule in each erythrocyte would double the turnover of iron.

A man with hypochromic iron-loading "sideroachrestic" anemia each day absorbed 3 mg. of iron from his diet and recycled 12 mg. for hemoglobin synthesis. There was no hemolytic disease. However, plasma iron turnover was 85 mg. per day. Only 10 per cent of injected Fe^{59} was incorporated into hemoglobin. The discrepancy between iron turnover and iron utilization for hemoglobin may represent recycling of siderocyte iron. In the patient's marrow most of the erythroblasts and many of the erythrocytes contained much stainable iron.

The patient was phlebotomized until he became moderately iron deficient. Now his marrow contained few sideroblasts and siderocytes. Because of blood loss his iron requirement for hemoglobin production was more than doubled (30 mg.) but plasma turnover was less than half (35 mg.), and 90 per cent of injected Fe^{59} was incorporated into hemoglobin.

These data may be indirect evidence of the recycling of siderocyte iron. Where the amount of siderocyte iron is great (as demonstrated by excessive iron in marrow sideroblasts or by high siderocyte counts after splenectomy) the recycling of such iron may result in an inflation of ferrokinetic values which hitherto have been interpreted as representing "ineffective erythropoiesis."

Respiratory Variations in Pulmonary Vascular Net Pressures. JEROME J. DALY, MASSIMO CALABRESI, ROBERT G. NIMS and FRANK D. GRAY, JR.,* New Haven, Conn.

Pulmonary vascular pressures as usually recorded fall with inspiration and rise with expiration. Inspiration produces an increase in the pressure gradient between the systemic veins and the right atrium leading to an increase in stroke volume and subsequent rise in pulmonary artery mean and pulse pressures. Finally, respiratory fluctuations of pulmonary artery blood pressure may arise from a change in vessel caliber and most evidence on this point supports the view that inspiration is associated with a fall in pulmonary vascular resistance. In this study the effects of changes in respiratory rate and depth on net pulmonary vascular pressures have been measured. Normal subjects and patients with pulmonary fibrosis or emphysema were studied.

Intraesophageal pressure was recorded by means of a water filled polyethylene tube attached to a statham strain gage. Gross vascular pressures were obtained by the technique of right heart catheterization. Net vascular pressures were obtained by feeding the outputs from these gages through adjustable resistances into a third amplifier. This system was stable over an adequate range of positive and negative pressures.

The effects of changing respiratory rate and depth on net right atrial pressure were qualitatively similar in all the subjects studied. With quiet respiration net atrial pressure rose by 3 to 5 mm. Hg during inspiration, falling with the succeeding expiration. At moderately rapid rates the inspiratory increase in net pressure averaged 6 to 10 mm. Hg. The maximum pressure rise of 10 to 15 mm. Hg occurred with rates down to seven per minute at maximum depth. At normal and moderately rapid rates of breathing there was no time lag between fall of intrathoracic pressure and rise in net atrial pressure.

Changes in pulmonary artery net pressures were most marked with increasing depth and decreasing rate of respiration. Pulse and mean pressures rose throughout inspiration. The maximum pressure level usually persisted into the early part of the expiratory cycle, and this change was more apparent with increasing rates of breathing at increased depth. In the majority of subjects at normal and slow rates of breathing the increase of vascular pressure occurred *pari passu* with the fall in intrathoracic pressure. The minimum increase varied between 4 to 6 mm. Hg for both systolic and diastolic pressures; the maximum increase lay between 10 and 15 mm. Hg. Net right ventricular pressures showed the same qualitative changes with respiration.

Net wedge pressures decreased during the initial fall in intrathoracic pressure in early inspiration in three subjects and the subsequent rise extended into the expiratory phase. The rise in net wedge pressures which occurred in expiration appeared to be dependent on the preceding inspiration as determined by the changes occurring during breath-holding. Increasing depth of respiration at moderate or slow rates was not associated with a greater rise in net wedge pressures as compared with the changes during quiet respiration. However, the initial fall in pressures with inspiration was more marked with increasing depth of breathing.

The above results lend some support to the view that respiration *per se* may be associated with changes in pulmonary vascular resistance.

Corticosteroid Binding Globulin Activity in Body Fluids and in Fetal and Maternal Plasma. WILLIAM H. DAUGHADAY,* IDA KOZAK and OLIVER BIEDERMAN, St. Louis, Mo.

Corticosteroid binding globulin (CBG) is a plasma alpha globulin with high affinity for corticosterone and cortisol. CBG in 100 ml. of normal plasma can reversibly bind 20 to 30 μg. of cortisol. To determine the distribution of CBG in body fluids, equilibrium dialysis was conducted with 10 ml. of plasma (or fluid), 0.5 μg. of cortisol-4-C^{14} and 80 ml. of phosphosaline buffer at 4° C. The percentage of unbound cortisol in the plasmas from men and nonpregnant women was 1.1 (0.7 to 1.5) (mean and range); three synovial effusions, 1.9 (1.3 to 2.9); four pleural effusions, 7.8 (3.8 to 16); seven spinal fluids, 93 (77 to 100).

The percentage of unbound cortisol was not decreased in five plasmas from pregnant women (mean, 2.1; range, 1.5 to 2.9), but was increased in six umbilical cord plasmas (mean, 16.2; range, 9.3 to 24). The unbound cortisol in three amniotic fluid samples was high (mean, 71; range, 60 to 89 per cent).

Normality of CBG in pregnancy was also demonstrated by double equilibrium dialysis in which plasma from pregnant women was directly compared to control plasma in the same environment of diffusible steroids of all types. Five ml. of maternal plasma and 5 ml. of plasma from a

nonpregnant subject in separate dialysis bags were dialyzed in the same tube against 80 ml. of buffer containing 0.5 μg. cortisol-4-C^{14}. The distribution of protein bound cortisol-4-C^{14} in the two plasmas at equilibrium is proportionate to the relative concentration of CBG (cortisol binding by other proteins is small under these conditions). CBG concentration in plasma from five pregnant women was 1.08 (mean), 0.93 to 1.36 (range) times the control. A similar examination of 12 plasmas from patients following intensive treatment with estrogens indicated that the CBG concentration was 1.09 (mean) (0.86 to 1.36, range) times the control. Elevations of 17-OH corticosteroid in these states are not attributable to CBG but may be related to other binding proteins.

The Transport and Synthesis of Lipid by the Small Intestine. ANTHONY M. DAWSON, VIRGINIA M. BELL and KURT J. ISSELBACHER, Boston, Mass. (Introduced by Marian W. Ropes).

When long chain fatty acids are absorbed from the gut they appear in the lymph as neutral fat. This transformation, which is an essential step in their absorption, has now been demonstrated *in vitro* by measuring the incorporation of palmitate-1-C^{14} into neutral fat by cell free homogenates of rat and human small intestine. This reaction requires adenosine triphosphate and coenzyme A, and is an enzymatic process distinct from lipase.

When palmitate-1-C^{14} is incubated with slices of intestine in the absence of albumin, it predominantly adheres to the cell surface and there is only negligible transport into the cell. If slices are incubated with an albumin-palmitate complex, mucosal transport of fatty acid with conversion to neutral fat can readily be demonstrated. These phenomena are strikingly stimulated by the presence of taurocholic acid—as much as fourfold in lower mammalian species and eightfold in man. Furthermore, the maximal taurocholate effect occurs even in the absence of albumin. This stimulation has not been observed with glycocholate and indeed the free bile salts, cholate and desoxycholate, have a pronounced inhibitory effect.

These observations emphasize the relationship between the transport and metabolism of fatty acids in the small intestine and suggest a probable role of a conjugated bile salt at the mucosal level. In contrast, free bile acids inhibit not only intestinal fatty acid metabolism but have also been shown to suppress the active transport of D-glucose and L-valine by the intestine.

In Vitro Calcium-Mobilizing Activity of Parathyroid Extract (PTE). WILLIAM P. DEISS * and LEILA B. HOLMES, Indianapolis, Ind.

Fresh paired rat femurs were incubated at 37° C. in media composed largely of human serum in an atmosphere of 95 per cent O_2–5 per cent CO_2. Fifty units of PTE were added to the experimental flasks and saline or formaldehyde-inactivated PTE at the appropriate pH was added to each control flask. The media were analyzed for calcium at the following times of incubation: zero, 24, 48 and 72 hours. In each experiment the control group's media calcium fell progressively throughout the study. Parathyroidectomized (PTX) rat bones extracted more calcium from the control media than did pretreated (PTE administered for 48 hours before sacrifice) PTX rat bones, and the latter bones extracted more than did normal bones. In each of these three groups, normal, pretreated PTX and PTX, the media with PTE added contained significantly more calcium than did the controls at 48 and 72 hours. In each experimental group there was an initial fall in medium calcium for 24 hours comparable to the controls followed by a progressive increase at 48 and 72 hours in the normal and PTX groups and by no further decrease in the pretreated PTX group. In each group at 48 and 72 hours the media pH was lower in the PTE experiments than in their control mates. At 72 hours the media phosphorus was lower in the PTE experiments.

These studies demonstrate the direct action of parathyroid extract on bone and illustrate a possible model system for more refined studies with purified parathyroid hormone of the mechanism of this action. The 48 hour lag in the calcium-mobilizing effect and the failure to demonstrate an increase in medium citrate in the one experiment where it was assayed are unexplained.

Effects of Vasopressin on Electrolyte and Water Metabolism in Primary Aldosteronism. JOSEPH F. DINGMAN, EDUARDO GAITAN and MAX C. STAUB, New Orleans, La. (Introduced by George E. Burch).

A patient with hyperaldosteronism manifested polydipsia, polyuria, hyposthenuria and decreased concentrating ability with dehydration sufficient, however, to lessen polyuria. Subcutaneous Pitressin® (vasopressin) during hydropenia and daily intramuscular Pitressin® tannate increased Na excretion, urinary Na to K ratio and creatinine clearance and accelerated water reabsorption (negative C_{H_2O}). Intravenous adrenocorticotropic hormone (ACTH) administered during Pitressin® therapy augmented these changes. The increased Na and decreased water excretion lowered serum Na and osmolality to normal. Low Na with normal or high K intake decreased Na excretion, lowered serum Na to normal, lessened polyuria and resulted in negative C_{H_2O}. Low Na, high K intake raised serum K to normal, relieved muscle weakness but skeletal muscle biopsied during removal of the aldosteroma showed low K, high Na content at variance with normal serum values. Preoperative constant water diuresis study revealed: *1*) Normal diuretic response (U_{osm} 60); *2*) Definite antidiuretic responses to 50 mU intravenous vasopressin (U_{osm} *232*) and hypertonic saline (U_{osm} 244); *3*) Moderate antidiuretic response to 1.0 mg. nicotine intravenously (U_{osm} 133); *4*) Moderate natriuresis during nicotine antidiuresis and marked natriuresis (1,700 μEq. per minute) during hypertonic saline infusion; *5*) Increased K excretion and C_{cr} during endogenous vasopressin secretion (nicotine, hypertonic saline). Postoperatively, urine flow decreased

(negative C_{H_2O}), response to hydropenia improved, and preoperative electrolyte effects of Pitressin® during hydropenia were not apparent. Four months postoperatively, normal antidiuretic responses to 15 and 50 mU vasopressin and to hypertonic saline, but no response to 2.0 mg. intravenous nicotine, were observed. These data suggest that certain disturbances in electrolyte and water metabolism observed in this disorder may be associated with alterations in secretion or peripheral action of neurohypophyseal hormones.

The Circulation in Induced Acute Head Injury. JOSEPH T. DOYLE, DAVID W. RICHARDSON and EDITH L. HARDIE, Albany, N. Y. and Richmond, Va. (Introduced by John L. Patterson, Jr.).

Advantage has been taken of procedures in the processing of cattle in the abattoir to study the circulatory responses to severe cranial injury inflicted by a sledgehammer. Laceration of the meninges and scattered small hemorrhages variously located in the brain resulted. Twelve cows (*Bos taurus*) were studied. The following were recorded: respiration with a nasal tube, intravascular pressures with polyethylene tubing and strain gages, the electrocardiogram (ECG), cardiac output by indicator-dilution, and blood gas tensions by bubble equilibration.

Immediately following the blow sufficient to render the animal comatose, respiration ceased. Apnea persisted for a minute or more in some animals, followed by gradual and irregular return of breathing, while in others apnea was permanent. Immediately after head injury, heart rate rose from approximately 80 to 120 to 160 beats per minute. Carotid, pulmonary arterial and right atrial pressures also rose, the carotid strikingly (peak, 320 per 192 mm. Hg). In animals with return of respiration carotid and other pressures stabilized near control levels, whereas with permanent apnea pressures remained elevated until a gradual decline developed after 10 to 12 minutes. Cardiac output (C.O.) doubled in one animal within the first minute after a single blow to the head and, in another, C.O. fell to 45 per cent of control two minutes after two hammer blows. The ECG frequently developed ST depression and T inversion after injury with subsequent A-V conduction disturbances. Arterial P_{O_2} in one animal fell to 15 mm. Hg and P_{CO_2} rose to over 115 mm. Hg.

The results demonstrate marked vulnerability of respiratory function to head injury as compared with circulatory function. There is a critical period during which the respiratory center must recover from the immediate shock of the injury and reinstitute breathing, or, together with the rest of the brain and body, be irreversibly caught by a falling arterial O_2 and rising CO_2 tension.

Water Diuresis Following the Ingestion of Large Volumes of Slightly Hypertonic Urea Solution by Hydropenic Subjects. LAURENCE E. EARLEY, Boston, Mass. (Introduced by Maurice B. Strauss).

It has been suggested that the "osmotic" regulation of antidiuretic hormone (ADH) release depends upon some function (stretch, deformation, pressure) of cell volume in the receptor area. Thus the failure of hypertonic urea solutions, unlike sodium solutions, to terminate a water diuresis is ascribed to the fact that the urea traverses cell membranes with ease and hence does not decrease intracellular volume. If this is indeed the case, then the ingestion of a large volume of isotonic or slightly hypertonic urea solution (with a volume of distribution similar to that of solute-free water) should be as effective as a similar volume of plain water in inhibiting ADH release and initiating diuresis.

Accordingly, four hydropenic subjects whose mean serum osmolality was 284 ingested 2 L. of a 300 mOsm. solution of urea in water, which increased serum osmolality slightly to 287. A prompt "water" diuresis ensued, with an average peak urine flow of 14.7 ml. per minute and mean urinary concentration decreasing from 844 to 103 mOsm. Free-water excretion (C_{H_2O}) averaged 9.6 ml. per minute. The high osmolal clearance (largely urea) precluded maximal urinary dilution. Alcohol administration (51.6 Gm.) did not enhance significantly the free-water excretion resulting from the ingestion of 2 L. of 300 mOsm. urea solution.

The same quantity of urea taken in a small volume of water produced increases in osmolal clearance similar to those after more dilute solutions but was not accompanied by free-water excretion.

It is concluded that the ingestion of large volumes of slightly hypertonic urea solution by hydropenic subjects results in a suppression of ADH release of the same order of magnitude as expected after the ingestion of an equal volume of solute-free water.

Hemodynamic Effects of Epinephrine in the Presence of Ganglionic Blockade in Conscious Dogs. JOHN W. ECKSTEIN and A. W. HORSLEY, Iowa City, Iowa. (Introduced by E. L. DeGowin).

Five large dogs were paralyzed with gallamine triethiodide and ventilated with a fixed-volume respirator. Ventilation was adjusted initially so that end-expiratory CO_2 tension was about 30 mm. Hg. Mean airway pressure was approximately 2 mm. Hg. The autonomic nervous system was highly responsive since touching the animals lightly caused marked fluctuations in pulse rate and blood pressure. Studies were done with dogs in the 15 degree head-up position. Right atrial and carotid arterial pressures and end-expiratory CO_2 tension were recorded continuously. Timed injections of indocyanine green dye were made into the right atrium and curves were registered from the carotid artery with a densitometer. Cardiac output and "central" blood volume were calculated by Hamilton's methods. Dye curves were obtained before and within 10 minutes after intravenous injection of hexamethonium bromide (50 mg.), and during intravenous infusion of epinephrine chloride (1 mg. per Kg. per minute) while the hexamethonium-induced ganglionic blockade continued.

Arterial pressure, heart rate, end-expiratory CO_2 tension, cardiac output and central blood volume regularly decreased after hexamethonium and regularly increased (usually to more than control values) during epinephrine infusion. Right atrial pressure fell in four experiments and remained unchanged in one after hexamethonium. Stroke volume fell in four experiments and increased slightly in one after hexamethonium and regularly increased to greater than control values with epinephrine. Cardiac output and central blood volume averaged 4,284 ml. per minute and 792 ml., respectively, during control periods, 2,233 and 623 after hexamethonium and 4,980 and 1,070 with epinephrine.

Reductions in central blood volume, cardiac output and right atrial pressure after hexamethonium administration are consistent with the hypothesis that hexamethonium releases venous tone and permits redistribution of blood peripherally. Restoration of these functions with epinephrine, a potent peripheral venoconstrictor, supports this suggestion.

The Synthesis and Turnover of Oxalic Acid in Normal and Hyperoxaluric Subjects. T. DAVID ELDER and JAMES B. WYNGAARDEN,* Durham, N. C.

Excretion, synthesis and turnover of oxalate were studied in six normal and three hyperoxaluric subjects. In normals, urinary oxalate was 10 to 45 mg. per day, and was promptly labeled following ingestion of glycine-1-C^{14}. In one subject, labeling of urinary hippurate was parallel to but twice that of oxalate, indicating a small dilution between glycine and oxalate pools. Oxalate-C^{14} given intravenously did not label expiratory CO_2, and 89 to 97 per cent was recovered in urine within 36 hours. The miscible pool of oxalate ranged from 4.9 ± 0.7 (S.D.) to 8.6 ± 1.1 mg., and its turnover from 34.0 ± 6.7 to 63.8 ± 11.3 mg. per 24 hours which in each case included the urinary oxalate value in the range (mean $\pm$ 2 S.D.).

Three subjects with hyperoxaluria and recurrent calcium oxalate stones were studied. Two received glycine-1-C^{14} and showed enrichment of urinary oxalate lower than controls. Urinary oxalate elevations were inconstant, declining in one subject from 110 to 41 mg. per day spontaneously, and in another from 117 to 11 mg per day during dietary protein restriction to 35 Gm. per day. Before protein restriction the pool was 39.1 ± 2.4 mg., and its turnover 109.7 ± 7.8 mg. per day. This pool was six times normal and correction for excess dilution within this large oxalate pool indicated over-incorporation of glycine-1-C^{14} into oxalate in this patient, despite the low enrichment values of urinary oxalate. When urinary oxalate had declined to 11 mg. per day, the pool was 1.8 ± 1.0 mg., and its turnover 8.2 ± 3.8 mg. per 24 hours.

Hyperoxaluria apparently results from overproduction of oxalate from glycine, probably via glyoxylate, and may be accompanied by gross expansion of the miscible oxalate pool. The excess oxalate produced from glycine might result from underactivity of either the glyoxylate dehydrogenase or glyoxylate transaminase systems. The inconstancy of hyperoxaluria in two patients suggests that cofactor, inhibitor and protein load influences must be considered in addition to a possible primary enzymatic deficiency.

"Cerebral Salt-Wasting": An Example of Sustained Inappropriate Release of Antidiuretic Hormone. FRANKLIN H. EPSTEIN * and HOWARD LEVITIN, New Haven, Conn.

The paradoxical association of hyponatremia and renal salt-wasting in patients with neurological disorders has been reported previously. A 19 year old girl experienced several episodes of lethargy, confusion and convulsions over a period of three years. Serum sodium ranged between 115 and 125 mEq. per L. at the same time that she excreted over 100 mEq. of sodium daily in the urine. The electroencephalogram was grossly abnormal. Treatment with desoxycorticosterone acetate (DCA) effected only temporary retention of salt and did not restore serum sodium to normal. Restriction of water while on a sodium-free diet induced a rise in serum sodium from 125 to 138 mEq. per L., accompanied by a loss of body weight. At the same time, renal excretion of sodium progressively diminished to less than 2 mEq. daily, only to increase again when drinking of water was encouraged. Water diuresis was grossly impaired at both low and normal levels of serum sodium and was not improved by chlorpromazine, benactyzine or pretreatment with large doses of prednisone. Ingestion of alcohol resulted in a slight increase in urine flow and fall in urinary osmolality but not to hypotonic levels.

Two hours after drinking 1,500 ml. of water the patient was venesected, in an attempt to demonstrate circulating antidiuretic hormone. Infusion of 350 ml. of her plasma into a patient with diabetes insipidus produced a transitory decrease in free water clearance and a small rise in urinary osmolality.

Menses, thyroid function (BEI) and insulin tolerance are normal. Plasma cortisol rose normally in response to insulin hypoglycemia. On water restriction her serum sodium has remained normal and the electroencephalogram has improved, although inability to excrete administered water persists. Except for this abnormality, renal function appears normal.

The patient appears to represent an example of hyponatremia and renal salt-wasting secondary to the sustained and inappropriate release of antidiuretic hormone.

Maturation and Multiplication of Nucleated Red Cells In Vitro. ALLAN J. ERSLEV * and JAMES R. HUGHES, Boston, Mass.

Three stages can be recognized in the transformation of stem cells to mature red cells. First, multipotential stem cells are differentiated to pronormoblasts, a nonmitotic process which probably is controlled by the erythropoietic factor. Second, nucleated red cells go through a number of mitotic divisions, and third, maturation and hemoglobin synthesis take place.

In order to study the dynamics of each of these stages iron incorporation was measured in a suspension of

rabbit bone marrow with and without added colchicine. Normal marrow was suspended in serum, Fe^{59} was added and the mixture incubated in a Dubnoff incubator at pH 7.4, 40° C., and an atmosphere of 13 per cent O_2, 5 per cent CO_2. Samples were removed after four, eight and 21 hours, and total iron uptake determined.

Iron incorporation at four hours was expressed in micrograms per immature red cell (nucleated cells + reticulocytes) and used as a measure of hemoglobin synthesis (normal, 1.35×10^{-9} μg. per cell per hour). The difference in iron incorporation at 21 hours in aliquots incubated with and without colchicine was used as an index of mitotic activity (normal, 35 per cent).

It was found that: *1*) Nucleated red cells incorporate slightly more iron than immature reticulocytes but much more than mature reticulocytes. *2*) Marrow suspended in "anemic serum" shows the same rate of hemoglobin synthesis and multiplication as marrow suspended in normal serum. *3*) Hemoglobin synthesis and cell multiplication increase with increasing PO_2 and depend critically on a physiologic pH and PCO_2. *4*) Normal marrow suspended in uremic serum shows a depression of hemoglobin synthesis but not of cell multiplication.

A technique has been developed suitable for the study of hemoglobin synthesis and red cell multiplication, but probably not suitable for the study of stem cell differentiation and action of erythropoietic factor.

Fucose and Blood Group Substances in the Salivas of Duodenal Ulcer Subjects. DAVID A. PRICE EVANS, Liverpool, U. K. (Introduced by Victor A. McKusick).

It has been suggested that the greater susceptibility of ABH nonsecretors to develop duodenal ulcer is due to their duodenal mucosae being protected by smaller concentrations of blood group mucoids than are the duodenal mucosae of ABH secretors.

Analytical data are known for the pure blood group active mucoids A, B, H and Le^a. They all contain L-fucose.

The concentration of L-fucose in saliva has been determined in 472 subjects, 332 of whom were free of peptic ulcer symptoms and 140 of whom had duodenal ulcers. The blood group and ABH secretor status of these subjects is known.

There is shown to be a significant correlation between $\log_{10}$ fucose concentration and the agglutination-inhibition titer of a number of specificities. In view of these correlations, and of the facts known about the distribution of fucose in the body, it seems that the fucose concentration of saliva is an index of its total blood group substance concentration.

Nonsecretors of ABH substances have almost as much fucose in their salivas as ABH secretors. This is ascribed to Le^a substance. It is computed that the mean total blood group substance concentration is of the same order in all phenotypes.

Duodenal ulcer subjects from two phenotypic classes have the same salivary fucose concentrations as do nonulcer subjects.

These findings indicate that the susceptibility of ABH nonsecretors to duodenal ulcer is not due to their having lower concentrations of blood group substances in their body fluids; and that persons who have developed duodenal ulcers have not done so because they are secretors of blood group substances in weak concentrations.

The Antigenicity of Insulin. CALVIN EZRIN and PETER J. MOLONEY, Toronto, Canada. (Introduced by R. F. Farquharson).

Direct evidence for the existence of neutralizing antibodies to insulin was obtained from a study of the serum of a horse which had been given a series of injections of crystalline ox insulin. At a certain stage in the course of inoculation, serum specimens showed insulin-neutralizing property (inhibition of insulin-induced convulsions in mice). At a later stage the serum was not only capable of neutralizing insulin but also showed flocculation on mixture with insulin. Further, from washed floccules both insulin and neutralizing anti-insulin could be obtained in good yield, indicating that neutralization and flocculation of insulin were due to one and the same antibody. It is most probable that neutralization of insulin by earlier serum specimens which did not show flocculation was also due to specific antibody.

We have studied sera from eight patients in whom neutralizing antibodies to insulin developed during treatment of diabetes. In one case studied in considerable detail clinical resistance was demonstrated to ox, pig and sheep insulins. This patient's serum prevented convulsions in mice ordinarily induced by ox, pig, sheep, horse, whale, chicken, fish(ling), monkey and human insulins; but it did not neutralize guinea pig insulin. This pattern of neutralization is the same as that exhibited by the antibody to insulin induced in the guinea pig, rabbit, sheep and horse by the repeated injections of ox or pig insulin. In none of these species did the antibodies to insulin neutralize endogenous insulin in the producers as evidenced by the presence of normal levels of blood sugar. Neither was the endogenous insulin of the patient neutralized by her own antibodies since it was possible finally to control the diabetes by diet alone.

Studies on the Mechanism of Action of Salicylates; Effects on Oxidative Phosphorylation. A. B. FALCONE, Madison, Wisc. (Introduced by Ovid O. Meyer).

The effect of sodium salicylate on adenosine triphosphatase (ATPase) activity and the P_i^{32}-ATP exchange reaction associated with oxidative phosphorylation in the respiratory chain has been investigated.

Intact rat liver mitochondria were incubated with P^{32} orthophosphate (P_i^{32}), ATP, KCl and appropriate quantities of sodium salicylate. ATPase activity during the incubation period was measured by determining increase of inorganic phosphate in the medium. The P_i^{32}-ATP

exchange reaction was determined by estimating the amount of P_i^{32} incorporated into ATP.

As a result of these investigations it was found that sodium salicylate at concentrations of the order of magnitude obtained in blood during therapy had a marked inhibitory effect on the P_i^{32}-ATP exchange reaction (5×10^{-4} M, 30 per cent inhibition; 1×10^{-3} M, 50 per cent inhibition; and 3×10^{-3} M, 80 per cent inhibition). ATPase activity was concurrently increased. Addition of dinitrophenol (DNP) at concentrations 1/100th of those for sodium salicylate also inhibited the exchange reaction and increased ATPase activity; however, the effect on ATPase activity for a given level of inhibition of the exchange reaction was much greater in the case of DNP.

Measurement of P/O ratios by others have demonstrated the uncoupling of oxidative phosphorylation by sodium salicylate. The results presented strongly indicate that the mechanism of this uncoupling probably resides in the transphosphorylation reactions associated with oxidative phosphorylation. Increased formation of P_i and ADP and decreased formation of ATP indicated by the results presented offer a rational explanation for the following effects of salicylates which have been noted by others: *1*) increase in BMR and *in vitro* increase in O_2 uptake by various tissues, *2*) lowering of blood glucose in some diabetic patients and accelerated glucose uptake by isolated diaphragm, and *3*) increased negative nitrogen balance, decreased prothrombin levels *in vivo* and decreased incorporation of amino acids into protein of muscle *in vitro*.

The Effect of Antigen Concentration on the Initiation of Antibody Synthesis in Rabbits. RICHARD S. FARR and FRANK J. DIXON,* Pittsburgh, Pa.

Circulating antibody is presumed to be instrumental in the pathogenesis of many allergic states; yet the conditions necessary to initiate antibody synthesis are not well understood. The present study was undertaken to learn what effect antigen concentration might have upon the amount of antigen needed to initiate antibody synthesis.

Rabbits were injected intravenously with from 6 to 8,000 μg. I^{131} labeled bovine serum albumin nitrogen (I*BSA N) or subcutaneously with from 0.001 to 5.0 μg. I*BSA N in 1 ml. of incomplete adjuvant. At least two of four criteria were used to determine whether each of the 220 rabbits studied had produced antibody: *a*) immune elimination phase in the I*BSA disappearance curve after intravenous test injections; *b*) detection of I*BSA-antibody complexes during immune elimination; *c*) demonstration of residual circulating anti-BSA *in vitro* or *in vivo* 20 to 30 days after the test antigen was injected; and *d*) the ability to produce an anamnestic response after reinjection of I*BSA.

Results indicate that no animals responded below a concentration of 0.05 μg. I*BSA N per ml., whether expressed as concentration of antigen in the 0.5 ml. saline compartment of 1 ml. of adjuvant, or whether expressed as concentration in the serum five minutes after intravenous injection. In both groups, the first antibody responses were initiated at concentrations of 0.1 μg. I*BSA N per ml. and approximately 50 per cent responded at 0.3 μg. per ml. Concentration was also a critical determinant for the initiation of antibody synthesis within the adjuvant group itself because multiple injections of dilute antigen were not nearly as effective as an equal total dose of concentrated antigen given in one place.

It is concluded that the concentration of antigen to which the antibody synthesizing cells are exposed is more critical to the initiation of antibody synthesis than is the total amount of antigen injected.

The Effect of Organomercurials on the Absorption of Na^{24} from the Human Small Intestine. JOHN T. FARRAR and VICTOR W. GROISSER, New York, N. Y. and Jersey City, N. J. (Introduced by Thomas P. Almy).

Because of possible similarities between renal tubular transport mechanisms and those of the small intestinal mucosa, the effect of an organomercurial compound on intestinal absorption of Na^{24} has been studied in man.

The absorption of isotonic $Na^{24}Cl$ (15 to 20 μc.) administered into the jejunum was calculated in 22 normal patients by integrating the arterial appearance curve of Na^{24} with the arterial disappearance curve of Na^{22} following simultaneous intravenous injection (10 μc.). Arterial blood samples were drawn at one minute intervals through a Cournand needle. The mean absorption rate was 91.6 per cent in 12 minutes. In nine of the subjects an identical test of Na^{24} absorption was performed (80 to 90 μc.) one hour after the first test. Significantly less variation in absorption rates was observed in the duplicate test in the same patient compared to that noted between different patients.

Using these data as a baseline, the absorption of Na^{24} was studied in eight additional patients before and after administration of r. rcaptomerin. Since the two tests in each patient were one to two hours apart, it was assumed that no appreciable change occurred in the sodium space. Therefore, Na^{22} was not used and simple Na^{24} arterial concentrations were determined. An indwelling catheter permitted determination of urinary flow and sodium and chloride concentrations. Mercaptomerin (2 ml.) was administered intravenously after the control test. Sixty to 100 minutes later, when a diuresis was observed, the second absorption study was performed.

Although a definite natriuresis and diuresis were observed following mercaptomerin, the rate of intestinal absorption of Na^{24} was unchanged in seven of the eight patients.

These studies suggest that: *1*) The technique of two closely spaced Na^{24} absorption tests in the same patient is a simple method for studying acute effects on sodium absorption, and *2*) intravenous organomercurials do not decrease the rate of intestinal absorption of Na^{24}.

An Unusual Case of Diabetes Mellitus with Increased Pancreatic Production of Insulin. JAMES B. FIELD, ARNOLD WEINBERG, PHYLLIS JOHNSON and STANLEY SPOONT, Bethesda, Md. and Philadelphia, Pa. (Introduced by F. D. W. Lukens).

A 16 year old female with uncontrolled diabetes for one year received up to 38,000 units of insulin daily in the hospital without any appreciable effect on her constant hyperglycemia and acetonuria. There was no evidence of allergy to insulin. Beef, pork and sheep insulin, phenethylbiguanide (DBI), metahexamide and hydrocortisone did not modify her insulin resistance. Her clinical condition remained essentially the same when all medication was stopped. As long as three months after her last insulin injection, insulin-like activity was detectable in her plasma equivalent to at least 40 mU per ml. by the rat hemidiaphragm assay (normal is less than 0.01 mU per ml.). Fasted mice injected with 1 ml. of her plasma all died in hypoglycemia. Insulin-I^{131} (0.25 unit) disappeared with a half-time of eight hours (normal, 20 to 30 minutes). Although most of the insulin-I^{131} had disappeared in 48 hours, there was no decrease in the plasma insulin-like activity, suggesting that she was producing large amounts of endogenous insulin. Despite the presence of at least 40 mU of insulin per ml. of plasma, she went into diabetic acidosis but recovered following 18,000 units of insulin, steroids, fructose and lactate. A repeated injection of insulin-I^{131} the next day revealed a half-time disappearance of two hours. When she was in acidosis her leukocytes incubated *in vitro* did not utilize glucose but did respond to 0.6 unit insulin per ml. with a normal glucose utilization. Despite the administration of over 1,000 units of insulin per day, she had repeated episodes of acidosis. Since she seems to be producing large amounts of insulin, it is suggested that her diabetes results from an apparent failure of muscle and adipose tissue to respond to insulin.

"Vitamin D-Resistant Rickets": A Disease Associated with Normal Renal Transport of Phosphate. MORTON H. FIELD and ERIC REISS, St. Louis, Mo. (Introduced by Hugh Chaplin, Jr.).

The basic defect in vitamin D-resistant rickets is now generally believed to be a specific abnormality of renal tubular transport of phosphate. The present studies were performed to determine whether the low tubular reabsorption of phosphate (TRP) is caused by a tubular defect or by secondary hyperparathyroidism. Two factors tending to decrease parathyroid function were studied (hypercalcemia and phosphate deprivation).

The TRP was measured before, during and after a four hour infusion of 15 mg. of calcium per Kg. In 12 affected patients from three families, calcium infusion was associated with a prompt increase of TRP from low to normal levels. After seven days' therapy with 500,000 units of vitamin D daily, the baseline TRP was increased to normal levels and was further increased by the infusion of calcium.

Unaffected siblings responded to initial calcium infusion with a very slight increase of the TRP. After administration of vitamin D, the baseline TRP was essentially unchanged and showed a moderate to marked decrease following the subsequent infusion of calcium.

The response of the affected patients to calcium infusion was characteristic of decreasing parathyroid secretion: namely, an increase in the serum phosphorus and in the filtered phosphorus load, associated with an increased TRP.

The affected patients and their unaffected siblings showed a normal response to phosphorus deprivation: namely, an increased TRP.

These observations strongly support the importance of secondary hyperparathyroidism in the etiology of vitamin D-resistant rickets and exclude an absolute intrinsic tubular defect of phosphate transport. An abnormal sensitivity of the renal tubules to normal parathormone appears to be unlikely since two affected children showed a normal response to exogenous parathormone (Lilly). Whether the basic defect is malabsorption of calcium or secretion of an abnormal parathormone is not certain.

Studies Using Anterior Pituitary Hormones as Antigens. J. FISHMAN, ELEANOR MCGARRY and J. C. BECK,* Montreal, Canada.

The development of new qualitative and quantitative immunochemical techniques have provided sensitive methods for identification and measurement of antigens. Recent reports on the production of antibodies to protein hormones for the most part suggest that such antigen-antibody systems have a high enough degree of specificity to be used to identify and measure these hormones.

In our laboratory intact rabbits received a course of injections with bovine and porcine growth hormone, adrenalectomized rabbits with porcine adrenocorticotropic hormone (ACTH), and thyroidectomized animals with beef thyroid-stimulating hormone (TSH). Hemaglutinating antibodies to beef growth hormone, pig growth hormone, ACTH and TSH have clearly been demonstrated using bisdiazotized benzidine to couple the antigen to the red cell. Preincubating the antiserum with the specific antigen inhibited the reaction. Each antiserum was preincubated with beef growth hormone, pig growth hormone, human growth hormone, pig ACTH, beef TSH, porcine pituitary gonadotropin and bovine gamma globulin. No cross reactions occurred with the antiserum to pig growth hormone. Anti-beef growth hormone serum showed some cross reaction only with bovine gamma globulin. Using the antiserum to pig ACTH the reaction could only be inhibited by ACTH and was inhibited by either human or pig ACTH. The antiserum to TSH showed cross reactions with porcine growth hormone, beef growth hormone, porcine pituitary gonadotropin and porcine ACTH but not with bovine gamma globulin. These results provide immunological evidence that the growth hormones tested are species specific but ACTH is not.

The smallest amount of specific antigen in 0.1 ml. which can consistently inhibit the reaction has been found to be 0.1 μg. in the case of beef growth hormone and 0.01 μg. in the case of pig growth hormone and ACTH. Preliminary attempts have been made to measure ACTH in human serum.

Further studies, including immunization of rabbits with human growth hormone, are in progress.

Pathogenesis of the Hemorrhagic Diathesis Developing during "Fibrinolytic" States: The Significance of Defective Fibrin Polymerization. ANTHONY P. FLETCHER,* NORMA ALKJAERSIG and SOL SHERRY,* St. Louis, Mo.

Hemorrhagic complications secondary to "fibrinolytic" states occur when a coagulation defect becomes superimposed upon a hyperplasminemic state. The pathogenesis of this defect has been investigated *in vitro*, and in patients with spontaneously occurring or induced "fibrinolytic" states.

Purified fibrinogen, partially digested by plasmin, clots slowly on treatment with thrombin and when added to normal fibrinogen inhibits clotting by thrombin. Antithrombin assay constitutes a nonspecific determination for this property. Electrophoresis during the early stages of fibrinogen digestion, when antithrombin activity is greatest, shows a characteristic deformation of the fibrinogen peak with evidence of fast running components. Plasma treated with plasminogen activators develops antithrombin activity; fractionation (Kekwick ether method) permits the demonstration in fraction F1 of antithrombin activity and the characteristic electrophoretic anomaly. Kinetic studies using fibrinogen substrate (dissolved in 1 M bromide at pH 5.3) or synthetic substrates reveal that digested fibrinogen does not inhibit the proteolytic action of thrombin but inhibits the subsequent steps in fibrin polymerization or gelation. The demonstration (electrophoretically) that fibrinogen breakdown products are actually incorporated into these clots supports the concept that the defect is explainable on a physicochemical basis. This view receives additional support from preliminary electron microscopic studies.

Hyperplasminemia in patients causes a fall in plasma fibrinogen and Factor V, but the accumulation of fibrinogen breakdown products and the development of defective fibrin polymerization is the more significant abnormality. Pilot survey suggests that the biochemical lesion of defective fibrin polymerization may be comparatively common and relevant to the hemorrhagic complications encountered in leukemia.

The Influence of Solute Load on the Isosthenuria of Renal Disease. STANLEY S. FRANKLIN, JOHN F. NIALL and JOHN P. MERRILL,* Boston, Mass.

Patients with both acute and chronic renal disease are characteristically unable to produce concentrated urine. If this defect is due to an overall reduction in renal mass so that each nephron is exposed to a greater solute load per unit time, one might expect improved concentrating ability by reducing the filtered load of solute. In order to test this hypothesis on patients with renal insufficiency two experimental methods were used: *1*) Glomerular filtration was reduced by producing hypotension with ganglionic blockade. *2*) Blood urea concentrations were substantially lowered by hemodialysis and jejunal perfusion. Four patients with renal insufficiency were subjected to induced hypotension. Despite reductions in osmolar clearance by an average of 58 per cent, osmolar U/P ratios with maximum Pitressin® (vasopressin) stimulation showed either a slight or no significant increase. In five patients undergoing dialysis for decompensated renal insufficiency serum urea was lowered by an average of 59 per cent. Despite this lowering in the filtered urea load, osmolar U/P ratios with maximum Pitressin® stimulation were unchanged.

These results suggest that the isosthenuria of renal disease may be due to an absolute reduction in free water reabsorption ($T^c_{H_2O}$). The dissociation between nitrogen retention and inability to concentrate urine in some early renal disease as well as the consistent depression of free water reabsorption in the most advanced renal disease may be explained by involvement of the functional and structural integrity of the medullary countercurrent concentrating area of the kidney.

The Utilization of Glucose and Metabolism of Insulin in the Exercised Extremity of Man. NORBERT FREINKEL,* CHARLES A. SANDERS, GILBERT E. LEVINSON and WALTER H. ABELMANN,* Boston, Mass.

Despite clinical evidence for diminished insulin requirements in exercising diabetic subjects, convincing demonstration has not been obtained that this is due to augmented glucose utilization nor has the role of insulin been defined.

Two types of studies were performed:

a) Four fasting normal subjects were given continuous intravenous glucose infusion designed to stop splanchnic glucose output and to continuously mobilize endogenous insulin. After two hours of blood sugar stabilization at 120 to 150 mg. per cent, leg exercises with a bicycle ergometer were instituted for 30 to 60 minutes. Glucose extraction by splanchnic viscera and by exercised and unexercised muscles was assessed before, during and after exercise from samples obtained simultaneously from brachial artery and cephalic, hepatic and femoral veins. During exercise, a fall in arterial glucose was accompanied by decreased arteriovenous (A-V) glucose differences in splanchnic area and arm, while these were unchanged or increased in the leg. Immediately after exercise, three- to fourfold augmentation of A-V glucose differences occurred in the exercised extremity and exceeded concurrent values in other areas for 15 to 30 minutes. Similar results were obtained following exercise in studies performed without infusion in two juvenile diabetics 24 hours after withdrawal of insulin.

b) T-1824 dye, as an intravascular reference standard, was mixed with I^{131}-insulin and injected into the right femoral arteries of five resting, normoglycemic subjects. Blood from brachial artery and both femoral veins was obtained at 5 to 20 second intervals and analyzed for dye and iodoinsulin. An average of 11 per cent more insulin than dye left the circulation during a single passage through the resting leg. In three subjects, these studies were repeated immediately following ergometer

exercise. Transcapillary loss of insulin was unaltered in two and minimally increased in one.

The data indicate that exercise disparately promotes glucose assimilation in exercised muscle and that the phenomenon does not depend upon changes in the systemic or local availability of insulin.

Detection of Pulmonary Arteriovenous Shunts Using Intravenous Injections of Kr^{85} and T-1824 Dye. HARRY W. FRITTS, JR., ALFRED HARDEWIG, DUDLEY ROCHESTER and JACQUES DURAND, New York, N. Y. (Introduced by Dickinson W. Richards).

Radioactive krypton (Kr^{85}), dissolved in water and injected intravenously, is almost completely cleared from the bloodstream in a single passage through the lungs. This property, recently demonstrated in our laboratory, has been utilized in the present study to detect shunts between the pulmonary artery and veins. Unlike previous methods, the technique differentiates these shunts from other channels allowing venous blood to reach the left heart. The procedure entails: *1*) injecting Kr^{85} and T-1824 dye intravenously; *2*) inscribing an arterial dye-dilution curve; *3*) collecting one arterial sample to the peak of the curve and another to the point of recirculation; and *4*) analyzing these specimens for Kr^{85} and dye.

The Kr^{85} reaching the systemic circulation equals that carried by shunts plus that carried by capillaries. Experimental evidence indicates that the former follows the dye curve, while the latter approximates a sloping straight line. With the contours of these components known, the flows through the two pathways can be determined by solving two equations relating the ratio of Kr^{85} and dye in the injectate to the ratios in the two blood samples. Because only ratios are measured, blood from sources other than the pulmonary artery dilutes the concentrations equally and thus does not affect the estimation of shunts.

Two patients with known hemangiomas of the lungs were shown to have shunts of 8 and 10 per cent, respectively. Six patients with chronic pulmonary emphysema had no shunts. In six patients with Laennec's cirrhosis, having arterial oxygen saturations as low as 83 per cent, no pulmonary arteriovenous shunts were demonstrated, suggesting that intrapulmonary hemangiomas were not implicated in this reduction.

Isolation and Quantitation of Lysolecithin from Lipid Extracts of Serum from Normal Humans and Patients with Pancreatic and Hepatic Diseases. EGIL GJONE, Baltimore, Md. (Introducted by A. Mendeloff).

Cephalins, lecithin and sphingomyelin are generally regarded as the main phospholipid constituents of normal serum. In the present work an additional phosphorus-containing component of lipid extracts of human serum has been isolated by the use of silicic acid column chromatography (Hirsch). This material has been identified as *lysolecithin,* from chemical analysis, staining properties, hemolytic activity and infrared spectrometry. A vapour phase chromatographic comparison of the fatty acids of lecithin and lysolecithin has also been carried out. Our results confirm the findings of Phillips, who, by the use of a chromatographic technique, claims to have isolated lysolecithin from lipid extracts of human serum.

Quantitative studies with this technique revealed in eight normal human sera the following values, expressed in mg. lipid phosphorus per 100 ml. serum: cephalins, 0.63; lecithin, 6.54; sphingomyelin, 1.79; lysolecithin, 0.88; or, expressed as per cent of the total recovered phosphorus, 6.4, 66.4, 18.4 and 8.9 per cent, respectively.

A quantitative study of lysolecithin in lipid extracts of serum from patients with acute and chronic parenchymal hepatic diseases, biliary atresia, and pancreatic disease has been carried out with this technique. Markedly elevated lysolecithin values were found in patients with some forms of pancreatitis and in some cases of biliary cirrhosis.

Abnormal Calcium Binding Associated with Hyperglobulinemia, Clotting Defects and Osteoporosis: A Study of this Relationship. HELEN I. GLUECK, RICHARD E. GOLDSMITH, LEO WAYNE and HELEN K. BERRY, Cincinnati, Ohio. (Introduced by Richard W. Vilter).

Bleeding and clotting abnormalities occur in disorders characterized by hyperglobulinemia (dysproteinemias). A patient with multiple myeloma, and one with hyperglobulinemia of unknown etiology who had severe proved osteoporosis, were found to have a delay in the rate of conversion of fibrinogen to fibrin by thrombin (thrombin time). Both bled abnormally and had water precipitable euglobins, cryoglobins and prolonged clotting times. Clots were bulky, and failed to retract normally in spite of normal platelets. Thromboplastin generation tests were normal. The second stage of clotting was not involved in the defect. The defect was present in whole and defibrinated plasma but was absent in serum. The abnormal thrombin time was corrected by the *in vitro* addition of an excess of either calcium or magnesium ions. The amounts required were far greater than the amounts known to shorten the thrombin time of normal plasma. The concentration of calcium in plasma in these patients was greater than that in serum. This difference could be accounted for by calcium analysis of the euglobulin fraction. Binding of calcium by the abnormal gamma globulin was further demonstrated by a calcium-staining dye applied to paper electrophoresis strips. This was not observed in normal or in other hyperglobulinemic patients. Ultrafiltrable calcium content of each plasma, before and after precipitation of euglobulins, was less than normal, also indicating abnormal calcium binding. The intravenous infusion of ionic calcium, in amounts sufficient to normalize the serum ultrafiltrable calcium concentration, improved the thrombin time, corroborating the *in vitro* observations.

Bivalent ions are essential for final fibrin formation. Evidence is presented that calcium was bound *in vivo* in sufficient amounts by abnormal proteins to alter the thrombin time. The resulting alterations in calcium concentration were probably responsible for the clotting de-

fects. These alterations, plus abnormalities in the matrix, probably produced the osteoporosis.

The Effects of Norepinephrine and Epinephrine on Unesterified Fatty Acid Metabolism. ALAN GOLDFIEN and RICHARD J. HAVEL, San Francisco, Cal. (Introduced by Julius C. Comroe, Jr.).

In previous studies in man and dogs the intravenous injection of norepinephrine (N) and epinephrine (E) produced similar increases in plasma unesterified fatty acids (UFA), whereas hexamethonium administration produced a fall. The injection of these substances during constant infusions of palmitic acid 1-C^{14} (PA 1-C^{14}) indicated that these changes reflected alterations in the rate of addition of UFA to plasma.

In the following studies, fasting pentobarbital anesthetized dogs were injected intravenously with PA 1-C^{14} three hours after the administration of hexamethonium or the start of a six hour constant infusion of N or E (0.5 and 1.0 μg. per Kg. per minute). Analyses of plasma UFA, UFA radioactivity and expired O_2, CO_2 and CO_2 radioactivity were performed.

N produced a persistent and marked elevation of plasma UFA, while E caused transient elevations to a maximum level within an hour and a return to control concentrations in two hours. The difference may be related to the hyperglycemia (300 mg. per cent) observed during infusions of E. Hexamethonium administration was followed by a reduction in plasma UFA, fall in O_2 consumption and a decreased rate of appearance in expired air, of C^{14} given as PA 1-C^{14}. The infusion of N or E increased O_2 consumption, and the rate of decrease in expired $C^{14}O_2$ specific activity exceeded that observed after hexamethonium. The percentage of administered radioactivity expired in three hours was below that of untreated fasting dogs. The specific activity of $C^{14}O_2$ (cpm per mm.) one to three hours after PA 1-C^{14} injection frequently exceeded the corrected UFA specific activity (cpm per mm. per 17) in plasma obtained simultaneously, indicating that oxidation was not occurring directly from a pool having the specific activity of plasma during this period. These observations are consistent with the concept that the sympathetic nervous system has a tonic effect on the metabolism of UFA.

Catechol Amines in the Pulmonary Pressor Response to Acute Hypoxia. ROBERTA M. GOLDRING, GERARD M. TURINO, GERALD COHEN, A. GREGORY JAMESON and ALFRED P. FISHMAN,* New York, N. Y.

Acute hypoxia is known to elicit pulmonary arterial hypertension. The present study was designed to determine whether pressor amines contribute to, or potentiate, this process. For this purpose, the levels of norepinephrine and epinephrine in arterial and mixed venous blood were measured during the induction of pulmonary hypertension by acute hypoxia, infusion of norepinephrine and a combination of both. In nine subjects, cardiac catheterization and arterial cannulation were used to simultaneously record pulmonary arterial, "wedge" and systemic arterial pressures; the Fick principle and dye dilution curves were used to measure pulmonary blood flow and central blood volume. In two other subjects, direct measurements of pulmonary arterial, aortic and left atrial blood pressures were made following the injection of norepinephrine into the pulmonary artery during thoracotomy. The results indicate that: *1*) Acute hypoxia is not associated with abnormal levels of circulating catechol amines. *2*) Levels of infused norepinephrine five times greater than control are required to duplicate the pressor response to acute hypoxia. *3*) There is no pulmonary arteriovenous difference for norepinephrine. *4*) The pressor response to hypoxia involves an increased resistance to blood flow within the pulmonary vascular bed whereas the response to norepinephrine is secondary to an increase in left atrial pressure. *5*) There is no potentiation of the pressor effect of acute hypoxia by the infusion of norepinephrine. *6*) The pressor effects of hypoxia and norepinephrine are additive.

Hyperhemorrhea of Unknown Cause: A Possible Physiologic-Clinical Entity. RICHARD GORLIN,* NORMAN BRACHFELD, JOHN D. TURNER and JOSEPH V. MESSER, Boston, Mass.

Most "high cardiac output" states are explicable in terms of known shunt, hormonal or metabolic alterations. Holmgren recently described a high output state associated with subjective fatigue and palpitation, disappearing with physical training.

An elevated cardiac output without obvious etiology has been observed in seven patients seen initially because of cardiac murmurs and ECG evidence of ventricular enlargement. All were males between 17 and 48 years; one had associated identifiable heart disease and two had related symptoms.

Resting output was measured by three different techniques on 19 separate occasions in seven patients and averaged 6.2 L. per minute per $M.^2$ (normal, 3.3). Output did not change with sedation, responded variably on exercise but tended to decrease during sleep. Three patients had mild systolic hypertension; all showed a labile wide pulse pressure: 67 mm. Hg (normal, 50). Heart rate averaged 82 beats per minute, and peripheral vascular resistance averaged 720 dyne-sec-$cm.^{-5}$ (normal, 1,270).

Cardiac catheterization revealed uniformly high O_2 content in venous blood from various sites, narrow A-V O_2 difference and no evidence of left to right shunt. O_2 consumption measured at time of output averaged only 20 cc. higher than laboratory average of 150 cc. per minute per $M.^2$. Studies of thyroid function, thiamine deficiency, lactate, pyruvate, pH, pCO_2 and blood catechol amines were normal. While transient anxiety is a possible explanation, no other circulatory parameters reflected this.

Since high cardiac output occurred in association with cardiac murmurs and cardiac hypertrophy, this may represent a distinct physiologic-clinical entity, although causation between high output and other observed changes cannot be established as yet. The cause currently remains obscure.

Suppression of Uric Acid Synthesis in the Gouty Human by the Use of 6-Diazo-5-Oxo-L-Norleucine (DON). ARTHUR I. GRAYZEL and J. E. SEEGMILLER, Bethesda, Md. (Introduced by Robert S. Gordon).

Despite the fact that an overproduction of uric acid has been shown to occur in a substantial portion of patients with gout, treatment of tophaceous gout has so far been directed towards increasing the ability of the kidney to excrete uric acid by means of uricosuric agents. A pharmacological agent that suppresses purine biosynthesis would provide a more logical approach to the therapy of such patients. In recent years the search for chemotherapeutic agents against cancer has produced two drugs, azaserine and 6-diazo-5-oxo-L-norleucine (DON), which have been shown by *in vitro* studies to block purine biosynthesis. Buchanan has shown that these agents compete with glutamine in the enzymatic conversion of formyglycinamide ribotide to formylglycinamidine ribotide.

We have found that the administration of DON to gouty subjects over a five day period results in a signficant fall in both the serum urate levels and urinary uric acid excretion. Studies of the incorporation of glycine-1-C^{14} into urinary uric acid in these patients further demonstrated that this effect of DON was indeed due to a suppression of *de novo* uric acid synthesis. In short periods of study the reduction of both serum urate levels and the urinary excretion of uric acid occurred before the appearance of any toxic symptoms attributable to DON.

The practical value of such drugs in the management of gout must await further investigations of their therapeutic index. These agents, however, are designed to treat the primary cause of hyperuricemia in those gouty patients who overproduce uric acid and thus represent a rational approach to their therapy.

Studies on the Collapse of Endotoxin Defenses Following Hemorrhagic Shock. SHELDON E. GREISMAN, Baltimore, Md. (Introduced by Theodore E. Woodward).

On the basis of considerable experimental data, it has been proposed that "irreversible" hemorrhagic shock is secondary to systemic absorption of gastrointestinal bacterial endotoxin coupled with collapse of the endotoxin defenses. The therapeutic implications are apparent: reduce gastrointestinal endotoxin absorption, or attack the mechanisms leading to deterioration of the endotoxin defenses. Lack of data concerning the latter mechanisms prevents a therapeutic approach at this level. The present studies consider possible factors involved in collapse of bacterial endotoxin resistance.

Hemorrhagic shock was produced in paired unanesthetized rabbits (0.8 to 1.5 Kg.) by three aseptic cardiac bleedings spaced at 60 and 30 minutes, respectively. The cardiac punctures *per se* did not impair myocardial contractility. By indirect criteria, arterial blood pressure was lowered to 54 ± 6 mm. Hg; maximum bleed-out was 48 ± 5 ml. per Kg. After bleed-out, one of each pair of animals was selected at random for a nonsterile femoral artery ligation; two hours later retransfusion was performed via an ear vein. Four hours after retransfusion, animals without femoral trauma exhibited no increase in susceptibility to 200 μg. per Kg. *E. coli* endotoxin intravenously, the maximum dose uniformly tolerated by normal rabbits; animals with nonsterile artery ligation exhibited 200- to 1,000-fold increases in endotoxin susceptibility ($p < 0.001$), similar to that following two hours of shock at 54 mm. Hg with the Lamson reservoir technique. Despite sterile precautions, femoral wound cultures revealed gram-positive anaerobes, and endotoxin susceptibility increased. Topical sulfanilamide applied immediately after "sterile" femoral artery cutdown prevented increased endotoxin susceptibility ($p < 0.01$), but not if applied two hours later or after artery ligation. Increases of 12 ± 10 Gm. per Kg. of the femoral ligated limb over the opposite control appeared within 24 hours of retransfusion.

It is concluded that a femoral wound created for bleeding participates in rabbit endotoxin defense deterioration following hemorrhagic shock. Contamination with anaerobic gram-positive bacteria and local plasma transudation appear to be major contributing factors.

Effect of Ethanol Administration upon Serotonin and Norepinephrine Levels in Rabbit Brain. DEHA GURSEY, JOHN W. VESTER and ROBERT E. OLSON,* Pittsburgh, Pa.

The possible importance of serotonin and norepinephrine as neurohumoral agents in brain tissue led us to study the effect of ethanol upon the concentration of these agents in the brain stem of rabbits. Forty-eight rabbits were given 2 Gm. per Kg. of ethanol intravenously in normal saline and sacrificed after intervals of 30 minutes to 144 hours. The levels of serotonin and norepinephrine in the brain stem were determined, respectively, by the spectrophotofluorometric methods of Bogdanski, Pletscher, Brodie and Udenfriend (J. Pharm. exp. Ther. 1956, **117**, 82) and Shore and Olin (J. Pharm. exp. Ther. 1958, **122**, 295). It was found that serotonin was markedly depressed from an average of about 0.45 μg. per Gm. to 0.20 μg. per Gm. one-half hour following the alcohol infusion, at which level it remained for two hours. Studies at later intervals showed that approximately 100 hours were required before initial levels were regained. The delayed return of serotonin to normal levels resembles the response to reserpine (Shore and co-workers, Ann. N. Y. Acad. Sci. 1957, **66**, 609). Norepinephrine level was not influenced by a single dose of alcohol. The chronic administration of alcohol daily for seven days, however, resulted in a 50 per cent decrease in both serotonin and norepinephrine in rabbit brain stem.

These findings led us to investigate serotonin metabolism in chronic alcoholics. Preliminary experiments have shown that following a 10 Gm. dose of DL-tryptophan, chronic alcoholics withdrawn from alcohol one week or more excrete less 5-hydroxyindoleacetic acid

than nonalcoholic control subjects. These data suggest that the conversion of tryptophan to serotonin may be reduced in some alcoholics, and if representative of brain metabolism could provide a constitutional basis for the greater susceptibility of these individuals to ethanol addiction under appropriate psychological stress.

Observations on the Course of Chronic Nonobstructed Pyelonephritis in the Rat. LUCIEN B. GUZE, BERNARD H. GOLDNER, SYDNEY FINEGOLD and WILLIAM HEWITT, Los Angeles, Cal. (Introduced by Samuel H. Bassett).

Studies of the pathogenesis of experimental pyelonephritis have been limited because of lack of a suitable model. Although such factors as ureteral obstruction, mechanical trauma, localized injury to medulla, preceding staphylococcal infection with resultant scarring, and desoxycorticosterone induced arteriolarsclerosis will predispose the kidneys of experimental animals to pyelonephritis, the investigator is frequently unable to distinguish between the effects of the predisposing injury and the induced infection. The few micro-organisms capable of initiating pyelonephritis in the "nonmanipulated" kidney usually cause rapid death of the experimental animal or the renal lesion becomes bacteriologically sterile and the disease does not progress. The present data describe the course of chronic, nonobstructed pyelonephritis in the rat.

Following the intravenous injection of a strain of enterococcus (*Streptococcus faecalis*) into normal rats, acute pyogenic infection of the kidney occurred. The course of this infection was studied at various intervals after bacterial inoculation. Using quantitative techniques, it was noted that the number of bacteria per kidney remained relatively constant during the period when the renal lesion was progressing from acute abscesses (one to three weeks) to the scarred lesion of chronic pyelonephritis (three to six months). Further observations revealed that the medulla was the most susceptible part of the kidney. Bacterial multiplication began in this area, and it was here that the earliest histologic lesions were noted.

The effects of ureteral obstruction on the course of this infection were studied. While no increased "trapping" of bacteria occurred in the obstructed kidney, the microbial populations reached higher levels in these organs as compared to the unobstructed kidneys.

"Ascending pyelonephritis" occurred in approximately 50 per cent of rats following the injection of enterococci into the bladder. Quantitative bacteriologic data, obtained in the study of this model, will be presented.

Erythrocyte Enzymes in Paroxysmal Nocturnal Hemoglobinuria. ROBERT C. HARTMANN and JOSEPH V. AUDITORE, Nashville, Tenn. (Introduced by Hugh J. Morgan).

Erythrocyte acetylcholinesterase activity (AChE) was markedly reduced in eight of 10 patients with paroxysmal nocturnal hemoglobinuria (PNH). Hemoglobin-free stroma showed a reduction in AChE activity similar to that of intact PNH red cells. In normal blood the top layer (immature) cells of a packed column of erythrocytes possessed more AChE activity than the bottom layer (mature) cells. In PNH both layers showed subnormal AChE indicating that the defect is present in immature as well as mature cells. In one patient both layers of cells possessed virtually no AChE activity.

The nature and significance of the erythrocyte AChE defect remains unsettled. There was no appreciable diurnal variation in enzyme activity nor was there any alteration during hemoglobinuric episodes. One of the two PNH patients with normal erythrocyte AChE was the only case in remission. Extensive *in vitro* and *in vivo* studies provided no evidence of any inhibitor of AChE in PNH cells. No activator for the depressed AChE in PNH cells could be demonstrated in normal red cells or in pernicious anemia erythrocytes whose enzyme activity was elevated following vitamin B_{12} therapy. In the past certain concepts regarding the physiological role of erythrocyte AChE have been derived from studies on normal cells in which the enzyme activity has been inhibited by drugs (*e.g.*, physostigmine). The physiological behavior of PNH cells was, however, different from that of drug-treated cells.

Enzymes of the erythrocyte glycolytic system were also studied. Glucose-6-phosphate dehydrogenase activity was somewhat higher both in top and bottom layers of PNH erythrocytes than in normal cell specimens or in acquired hemolytic anemia specimens with comparable reticulocyte concentrations. Similar observations were made on erythrocyte 6-phosphogluconic dehydrogenase activity in PNH. Phosphoglucomutase activity was slightly greater in PNH cells than in control cells.

The Influence of Elastin Removal on the Elastic Properties of Lung Preparations. BILL F. HEFLEY and JOHN A. PIERCE, Little Rock, Ark. (Introduced by Richard V. Ebert).

The elastic properties of the lungs are essential for normal ventilation. The available evidence suggests that surface active forces are important for the maintenance of lung elastic properties, but the effect of tissue factors is unknown. The purpose of this study was to investigate the influence of elastin on these properties.

The pressure volume diagram of the lungs was measured in anesthetized dogs before and after opening the chest. The lungs were excised and rendered essentially free of elastin by incubation with elastase. The pressure volume characteristics were again studied. The mean pulmonary compliance was 44 ± 14 ml. per cm. H_2O in eight intact animals, 47 ± 11 ml. per cm. H_2O after the chest had been opened, and 36 ± 11 ml. per cm. H_2O after the removal of lung elastin. In five preparations, the mean compliance under saline distension was $1{,}600 \pm 400$ ml. per cm. H_2O.

This demonstrates that removal of elastin failed to alter significantly the volume elastic properties with air inflation, but altered these properties markedly when the preparations were distended with saline. These findings

suggest surface active forces are of primary importance to the maintenance of normal pressure volume relationships in the lungs. They call for a revision of certain concepts about factors influencing lung elastic properties.

The Alveolar pCO_2 and pO_2 in Pregnant and Nonpregnant Women at Altitude. ANDRE HELLEGERS, JAMES METCALFE, WILLIAM HUCKABEE, GIACOMO MESCHIA, HARRY PRYSTOWSKY and DONALD BARRON, New Haven, Conn. (Introduced by C. Sidney Burwell).

The mean alveolar pCO_2 and pO_2 in end-expiratory samples of air were studied in a group of three pregnant and four nonpregnant subjects living at an altitude of 14,500 feet in Tuctu, Peru. All subjects had fasted and refrained from smoking or exercise for one hour prior to testing. During the next one and one-half hours, with the subjects under observation, three end-expiratory alveolar air samples were collected in previously evacuated 25 ml. tonometers and immediately analyzed for CO_2 and O_2 concentration by the method of Scholander. The pCO_2 and pO_2 were calculated from the barometric pressure which averaged 456 mm. at this altitude. The final value for each test was the mean for the three determinations, all of which fell within a range of 2 mm.

For the pregnant group the mean alveolar pCO_2 was 22.93 mm., with a range of 19.37 to 25.71 mm., while the mean pCO_2 was 59.01 mm., with a range of 54.92 to 62.70 mm. For the nonpregnant group the mean alveolar pCO_2 was 27.94 mm., with a range of 25.59 to 30.54 mm., while the mean pO_2 was 50.71 mm., with a range of 44.92 to 53.52 mm.

Our figures compare with a mean pCO_2 of 29.1 mm. and pO_2 of 50.5 mm. in a group of males in Morococha (Peru) at an altitude of 14,900 feet, studied by Hurtado and co-workers.

Our data demonstrate that the hyperventilation, characteristic of high altitudes, is further significantly augmented by pregnancy at altitude. Knowledge concerning the reduction of pCO_2 during pregnancy at sea level suggests that during pregnancy at altitude, hyperventilation due to a hormonal mechanism is added to the hyperventilation due to the altitude. This hyperventilation increases the alveolar pCO_2 of pregnant women to levels above those reached by hyperventilation of altitude alone.

The Antigenicity of Connective Tissue. PAUL HELLER, VINCENT YAKULIS and HYMAN J. ZIMMERMAN,* Chicago, Ill.

The observation that patients with acute disseminated lupus erythematosus (L.E.), a disease of the connective tissue, have circulating antileukocytic antibodies, including the L.E. plasma factor, led to a study of the immunologic relationship of connective tissue and leukocytes. During the course of this study, marked variations in the antigenicity of various connective tissue preparations were noted. The possibility was considered that the antigenicity of connective tissue extracts might be related to the age of the donor animal.

Neutral salt extract of tendons from the small muscles of the lower extremities of seven day old (subsequently called "young connective tissue," "Y.C.T.") and aged rabbits (subsequently called "old connective tissue," "O.C.T.") were prepared. Aliquots of these extracts in Freund's adjuvant were injected into guinea pigs for a total of 300 μg. of protein, or approximately 36 μg. of hydroxyproline. Thirty-four guinea pigs received "Y.C.T." and 34 "O.C.T." Ten days following the last injection, 25 of the 34 animals immunized with "O.C.T." had developed high titers of complement fixing anti-connective tissue antibodies and 19 had also antibodies to leukocyte cytoplasm at a higher titer than 1 to 8. The response to "Y.C.T." was markedly smaller. The difference was statistically significant ($p < 0.005$). "O.C.T." was also the stronger test antigen. There was no cross reaction with leukocyte nuclei. No anti-connective tissue antibodies were demonstrated in animals immunized with leukocyte cytoplasm. On Ouchterlony plates, antisera produced with "O.C.T." and "Y.C.T." reacted only with "O.C.T." and leukocyte cytoplasm. No precipitin lines were demonstrable with "Y.C.T." as test antigen.

There is in connective tissue an antigen capable of promoting the formation of antileukocytic antibodies. The chemical characteristics of this antigenic component are still incompletely explored.

The Hypocholesteremic Effect of Androsterone. LEON HELLMAN,* H. LEON BRADLOW, BARNETT ZUMOFF, DAVID K. FUKUSHIMA and T. F. GALLAGHER, New York, N. Y.

The production of androsterone, an androgen metabolite, is specifically reduced by deficiency of thyroid hormone. Treatment with androsterone causes a significant decrease in serum cholesterol and in this sense a steroid can simulate at least one action of thyroid hormone. The following observations were made: *1*) Measurements were made of the daily production of the two principal endogenous androgen metabolites, androsterone (A) and etiocholanolone (E). The conversion of exogenous testosterone-4-C^{14} to A and E was also determined in order to evaluate the peripheral metabolism of androgen. *2*) In myxedema, the daily production of A plus E was reduced to 1.0 mg. as compared with 4.5 mg. for euthyroid subjects. Androsterone constituted 44 per cent of the total of A plus E in the controls, but only 15 per cent in the myxedema group. The myxedema patients converted a similarly small fraction of labeled testosterone to androsterone in contrast with the euthyroid subjects. *3*) The reverse was observed in hyperthyroidism in that androsterone production, from both endogenous and exogenous androgen, was elevated. *4*) Triiodothyronine administration altered this defect in steroid metabolism in the myxedematous patients and restored an essentially normal metabolic pattern for both endogenous and exogenous androgen. In euthyroid subjects, triiodothyronine materially increased the production of androsterone. *5*) From these results, it was postulated that some of the peripheral actions of thyroid hormone might be mediated by andros-

terone. Androsterone (50 mg. per day, I.M.) was administered to four patients with myxedema, seven patients with hypercholesteremia, and five normocholesteremic subjects. A highly significant fall ($p < 0.01$) in serum cholesterol was observed in all three groups. The effect was most striking in the myxedema patients where androsterone caused an 18 to 40 per cent decrease in serum cholesterol.

The significance of the "thyromimetic" activity of androsterone goes beyond duplication and dissociation of the peripheral activities of thyroid hormone. It is the first example of specific therapy for a defect in the intermediary peripheral metabolism of a hormone by administration of the hormone metabolite produced in inadequate amounts. This hitherto unsuspected property of androsterone suggests that it may be the prototype of a new class of substances with potential advantage for lowering blood cholesterol.

Excessive Renal Tubular Reabsorption of Filtered Urea in Human Adrenal Insufficiency. A. GORMAN HILLS,* DAVID W. PARSONS, OTTO ROSENTHAL and IRIS KIEM, Philadelphia, Pa. and Miami, Fla.

The question was investigated by measuring simultaneously the renal clearance of urea (C_U) and of inulin (C_{In}) at varying rates of urine flow (16 clearance periods, four subjects) during acute adrenal insufficiency and in suitable control subjects (26 periods, nine subjects). Mean log $(U/P)_{inulin}$ for the two groups did not differ significantly. Regression of C_U/C_{In} on log $(U/P)_{inulin}$ for controls (the best-fitting straight line for a mass plot of all control clearance periods) closely approximated results of others (Chasis and Smith, J. clin. Invest. 1938, **17**, 347). The corresponding regression line for the experimental group lay significantly lower: The adjusted mean C_U/C_{In} for experimental subjects (0.412) minus that for controls (0.535) was −0.123, corresponding to mean increase of the percentile reabsorption of filtered urea of 12.3, or mean increase of 26 per cent over the percentage of urea normally reabsorbed.

Supporting data were obtained using the clearance of endogenous creatinine chromogen (C_{Cr}) as a reflection of glomerular filtration rate. C_U/C_{Cr} was measured in 20 clearance periods in five persons in adrenal insufficiency and in 42 periods in 12 controls. The regression line of C_U/C_{Cr} on log $(U/P)_{creatinine}$ again lay lower in experimental subjects than in controls. The difference between the adjusted mean clearance ratios was −0.172.

In both of two adrenal-deficient patients studied, cortisone acetate therapy raised the mean clearance ratio in terms of the perpendicular deviation of the ratios from the appropriate regression line previously constructed for normal subjects (or, equally, for those in adrenal insufficiency), in one case significantly. The data indicate that the specific tubular abnormality is correctable by cortisone therapy, not necessarily by direct hormonal effect upon tubular cells.

The Fatty Acid Composition of Adipose Tissue in Man. JULES HIRSCH, JOHN W. FARQUHAR, MALCOLM L. PETERSON and WILHELM STOFFEL, New York, N. Y. (Introduced by E. H. Ahrens, Jr.).

Numerous recent studies suggest that adipose tissue is central in the regulation of lipid and energy metabolism. To investigate this role a simple method for repeated sampling of adipose tissue has been devised. One ml. of saline is injected into the superficial panniculus of the buttock and aspirated into a 50 ml. syringe by vigorously retracting the plunger. This permits the withdrawal of 1 to 10 mg. of adipose tissue droplets. The lipid is then extracted for analysis by gas-liquid chromatography. Serum obtained by venipuncture is used for analysis of the fatty acid structure of cholesterol esters, triglycerides, phospholipids and nonesterified fatty acids.

Adipose tissue and sera of nine normal subjects were investigated. One hypercholesterolemic male was also studied during rigidly controlled dietary manipulations made possible by the exclusive feeding of liquid formulas. Specimens of adipose tissue from different anatomic sites obtained at autopsy and surgery have also been analyzed.

The analyses demonstrate that the described method of biopsy yields a representative sample, since various adipose depots have only minor changes in composition. This adipose lipid is over 99 per cent triglyceride which contains more than 35 different fatty acids of 10 to 22 carbon atoms in length with zero to six double bonds. However, C_{16} and C_{18} acids comprise 90 per cent of the total. The fatty acid profile of adipose tissue is distinct from that of each serum lipid group, indicating that it is not in simple equilibrium with any of these groups. In further contrast to all serum lipids, adipose fatty acid composition is surprisingly constant during dietary changes. Hence, with prolonged feeding of a fat-free diet the usual linoleic acid content of 9 to 11 per cent is maintained. Such fixed composition is particularly remarkable in the light of the known rapid turnover of adipose fatty acids.

Studies of the Effect of L-Triiodothyronine upon Bile Acid Formation in Man. M. E. HODES, R. B. FAILEY, JR. and E. BROWN, Indianapolis, Ind. (Introduced by Paul J. Fouts).

The bile acids have been shown to be the principal excretory products of cholesterol, and the chief acids found in man are cholic, chenodeoxycholic and deoxycholic. In view of the reciprocal relationship between serum cholesterol level and the degree of thyroid activity studies were undertaken measuring the effect of L-triiodothyronine (Cytomel®) upon the composition of biliary bile acid.

Studies were made over a 24 hour period. Seven patients were studied by intubation and bile obtained by duodenal drainage. One patient had postoperative drainage through a T-tube. Dosage of L-triiodothyronine varied from 200 to 400 μg. which was administered in a single dose. Hydrolyzed bile was separated on Celite columns, and the individual bile acids subsequently measured colorimetrically.

All patients studied showed a consistent fall in the

ratio of chenodeoxycholic to cholic acid. The maximal fall was noted two hours after administration of the drug. This appears to be one of the earliest demonstrable effects of thyroid hormone. Values had returned to or exceeded control levels at the end of 24 hours. No consistent or significant effect on deoxycholic acid excretion was noted. The fistula patient, in whom total bile acid output could be measured, showed a slight increase in cholic acid excretion and a somewhat greater decrease in chenodeoxycholic acid excretion.

This consistent pattern of bile acid excretion is contrary to that reported following thyroid administration in the rat. Increased cholic acid excretion is, however, seen in man following administration of unsaturated fatty acids so that in humans a selective excretion of cholic acid may represent the principal means of cholesterol and bile acid excretion.

Measurements of the Amount of Irradiation of Human Ovaries Incident to Administration of Radioactive Iodine (I^{131}). ROBERT E. HODGES, TITUS C. EVANS, JAMES T. BRADBURY and WILLIAM C. KEETTEL, Iowa City, Iowa. (Introduced by William B. Bean).

Fear of irradiation damage to the ovaries has restrained many clinicians from giving radioiodine to young women who are thyrotoxic. Since the actual danger was unknown we attempted to measure it.

We selected five young women who were scheduled to have tubal ligation. At varying intervals before the operation we gave each of them a small dose of I^{131} orally; the surgical procedure was done three, 15, 24, 48 and 76 hours later. At operation, we obtained specimens of venous blood, myometrium, rectus muscle and ovary. These were measured for radioactivity and in each instance, the blood contained the most I^{131} per gram, and the ovary, myometrium and rectus muscle smaller amounts. The rate of loss was identical from all tissues. The effective half life was 15 hours and declined rapidly thereafter. From the data we calculated the amount of irradiation which would have occurred to the ovaries of a thyrotoxic woman of average weight who was given a standard therapeutic dose of I^{131} and retained an average amount of it in her thyroid. Comparison of this value with estimates by the Atomic Energy Commission (J. Amer. med. Ass. 1958, **166**, 233) of the ovarian irradiation which occurs in diagnostic X-ray procedures shows that therapeutic I^{131} results in less ovarian irradiation than these accepted procedures (X-rays of hip and pelvis or of lumbar spine).

Nine Patients with Pulmonic Stenosis, Right-to-Left Shunt Through a Ventricular Septal Defect, and Right Ventricular Pressure above Systemic Pressure. J. I. E. HOFFMAN, ABRAHAM M. RUDOLPH and ALEXANDER S. NADAS, Boston, Mass. (Introduced by Charles A. Janeway).

The basic physiological difference between pulmonic stenosis with an intact ventricular septum (PS) and with a right-to-left shunt through a ventricular septal defect (PS, VSD, R→L) is that in the latter there is rapid pressure equilibration across the defect. As a result in PS, VSD, R→L systolic pressures in the right and left ventricles and aorta are similar and rise slightly or not at all after ectopic beats, right and left ventricular pressure tracings have similar shapes with almost parallel sides and flat or rounded tops, and ventricular ejection ends at about the same time in the two ventricles so that the second heart sound is single or narrowly split and the systolic murmur does not pass beyond aortic valve closure. By contrast, if the right ventricular systolic pressure is much higher than that in the systemic circulation and rises over 10 mm. Hg after ectopic beats, if the right ventricular pressure curve is triangular with a pointed top and if right ventricular systole is prolonged so that the second heart sound is widely split and the systolic murmur passes aortic closure, then the inference is usually made that the pulmonic stenosis is severe and the ventricular septum is intact.

It is important to know about an associated ventricular septal defect before operation for it influences both the prognosis and the operative approach to the pulmonic valve. The purpose of this paper is to present nine patients who, although meeting most of the criteria of pulmonic stenosis with an intact ventricular septum (in particular, having right ventricular pressures much above systemic pressures), still were proven later to have an associated ventricular septal defect. The diagnosis of this unusual group of patients is discussed with special reference to the shape of the right ventricular pressure curves, the role of indicator dilution curves and cineangiography, and the value of phonocardiography.

Studies on Estradiol Mediated Pyridine Nucleotide Transhydrogenase in Human Breast Cancer. VINCENT P. HOLLANDER, Charlottesville, Va. (Introduced by William Parson).

Estradiol-17β mediated pyridine nucleotide transhydrogenase has been found in some but not all specimens of human breast cancer examined. *In vitro* stimulation by estradiol of transhydrogenation between reduced triphosphopyridine nucleotide (TPNH) and diphosphopyridine nucleotide (DPN) in the presence of tumor supernatant fraction is shown to be inhibited by 2′ adenylic acid. This latter nucleotide is an effective inhibitor of pyridine nucleotide transhydrogenation by placental supernatant. The inhibition by 2′-adenosine monophosphate (2′ AMP) of the mammary system is taken as further evidence for the specificity of the *in vitro* estradiol effect.

Further study has revealed three exceptions for the hypothesis that patients with an *in vitro* sensitive tumor will derive benefit from oophorectomy or adrenalectomy. One postmenopausal patient with no evidence of steroid mediated transhydrogenase in lenticular metastases showed complete clearing of recurrent disease following adrenalectomy. A premenopausal patient exhibited significant *in vitro* sensitivity to estrogen in a primary breast cancer. Two years later lenticular metastases

appeared and assay of biopsied material failed to show significant steroid sensitivity in metastatic tissue. Oophorectomy was followed by prompt regression of the lenticular metastases. A postmenopausal patient with significant *in vitro* sensitivity in recurrent tumor died four months after adrenalectomy with definite tumor progression. Five patients have shown concordant behavior between *in vitro* estrogen sensitivity and clinical response. Possible factors responsible for the discordant results will be discussed.

The Cholesterol Lowering Effect of 1-[*p*-(β-*Diethylaminoethyoxy*)-*Phenyl*]-1-(*p*-*Tolyl*)-2-(*p*-*Chlorophenyl*) *Ethanol in Hypertensive Man.* WILLIAM HOLLANDER and ARAM V. CHOBANIAN, Boston, Mass. (Introduced by Robert W. Wilkins).

In view of the favorable effect of hypotensive therapy in lowering serum cholesterol levels, and the foreign reports that parenteral procaine, a known hypotensive, ethanolamine compound, is of benefit in atherosclerosis, it was decided to try ethanolamine derivatives in hypertensive subjects as to their effects on blood pressure and cholesterol metabolism.

Three oral products, procaine amide, 2-dimethylaminoethanol, and 1-[*p*-(β-diethylaminoethoxy)-phenyl)]-1-(*p*-tolyl)-2-(*p*-chlorophenyl) ethanol (MER-29) were administered to 35 hypertensive subjects intermittently over a period of two to eight months. Of these, only procaine amide had a significant hypotensive effect. However, the most consistent changes in serum cholesterol were produced by MER-29, which caused a significant reduction in 12 of 15 subjects. The reduction in serum cholesterol during MER-29 treatment averaged 38 ± 7 mg. per cent and was not accompanied by significant changes in serum lipid phosphorus. At doses of 750 mg. per day it was well tolerated symptomatically.

During MER-29 administration, isotopic studies of cholesterol metabolism were performed in three of the subjects. The "miscible pool" of cholesterol as calculated from the disappearance rate of intravenously administered C-14-cholesterol was reduced on the average by 38 per cent. The rate of appearance of labeled cholesterol in the serum following the intravenous administration of C-14-acetate was also reduced on the average by 30 per cent following MER-29 administration.

Thus, MER-29 appears to be an effective agent in lowering serum cholesterol as well as "body miscible pool" of cholesterol in hypertensive subjects. It also appears to interfere with the conversion of acetate to cholesterol in man as has been found by Blohm in animals. Although MER-29 also has a structural resemblance to chlorotrianisine, a synthetic estrogen, it had no feminizing effects. Therefore, it is not clear whether its hypocholesterolemic effect is related to its "estrogenic" or to its ethanolamine structure.

Multiple "Antibodies" to Cell Constituents in Systemic Lupus Erythematosus (S. L. E.) and Related Disorders. HALSTED R. HOLMAN, HELMUTH R. G. DEICHER and HENRY G. KUNKEL,* New York, N. Y.

Studies from many laboratories have demonstrated that the L.E. cell factor can be absorbed by isolated cell nuclei, and that γ-globulin can be identified on these nuclei by various techniques, including the use of fluorescent antibodies. Further studies indicate the presence of many serum factors in S. L. E. which react with different nuclear and cytoplasmic constituents of the cell.

The L.E. cell factor reacts specifically with nuclear nucleoprotein and requires both desoxyribonucleic acid (DNA) and histone for the reaction. Other factors have been identified which react with DNA or histone alone, but which appear unable to induce L.E. cell formation. The reaction with DNA has been demonstrated by agar diffusion, precipitin and complement fixation techniques.

The most frequently encountered reaction involving nuclear constituents has occurred with a material extractable from nuclei with buffers, the active component of which is not nucleoprotein, DNA, or histone. While reactions with DNA-containing substances have appeared almost exclusively in S.L.E., reactions with the buffer extract of nuclei have had a somewhat wider disease distribution, including certain cases of scleroderma, rheumatoid arthritis and unclassified dysproteinemia.

An even more frequent reaction has been found when a whole tissue antigen or various fractions of cytoplasm have been employed. Most cases of S.L.E., certain types of liver disease and occasional other disorders have demonstrated this reaction.

An individual serum may possess one or more of the serum factors; titers are usually highest during acute illness and diminish during remission.

All the serum factors are γ-globulins, and some have been isolated. They possess many properties of antibodies. Their reaction with the patient's own tissues supports the view that they are autoantibodies, but proof of this hypothesis remains incomplete.

Fetal Hematopoietic Tissue Transplantation Following Lethal Whole Body Irradiation in Dogs, and a Consideration of Its Clinical Use. D. M. HUME,* B. T. JACKSON, H. G. KUPFER, W. T. HAM and R. H. EGDAHL, Richmond, Va.

The use of adult bone marrow to repopulate the hematopoietic system after fatal doses of irradiation has the disadvantage that the marrow cells may react immunologically against the host. Fetal liver has been used as a source of hematopoietic tissue in mice to obviate the reaction of the graft against the host (Uphoff). No permanently successful blood cell homotransplant has yet been reported between unrelated pairs of dogs, however, despite many attempts. In the present report splenectomized mongrel dogs were employed as recipients and liver and spleen from unrelated canine fetuses were used as the donor tissues. A series of 34 dogs were exposed to 600 r. of total body irradiation given in one dose from a 1,000 KVP source at a distance of 2 M. In 12 dogs infusions of liver and spleen cell suspensions were given from three to 72 hours after irradiation, while 22 dogs received saline infusions and were used as controls. In

preliminary experiments comparisons were made between intra-arterial, intramarrow and intravenous routes of infusion. One dog with myelogenous leukemia was given irradiation and died 24 hours later. An infusion of four month fetal liver cells was given to one clinical patient with aplastic anemia in whom irradiation was not employed. The results were as follows:

1) Four of 12 dogs receiving irradiation and liver and spleen cell infusions are surviving and in good health from 24 to 228 days later. *2*) All 22 control dogs died from seven to 19 days after irradiation. *3*) The intravenous route of administration was superior to the other two. *4*) The dog with leukemia showed no detectable leukemic cells 24 hours after total body irradiation. *5*) The patient did not appear to benefit from fetal liver infusion, although viable fetal liver cells were found growing in the bone marrow.

It is concluded that fetal liver and spleen cell suspensions are capable of sustaining life after fatal whole body irradiation in the dog. Fetal liver cells which were infused intravenously in the human without reaction were recovered later in the bone marrow and appeared normal histologically.

The Infectivity and Interrelationships of 2060 and J.H. Viruses Among Volunteers. GEORGE GEE JACKSON,* HARRY F. DOWLING and WILLIAM J. MOGABGAB, Chicago, Ill.

Two viruses, 2060 and J.H., were isolated in monkey kidney cell tissue cultures from subjects with an afebrile coryzal illness. Both have been implicated in the etiology of the common cold. The present studies were undertaken to determine whether the viruses cultivated in tissue culture caused infection and clinical symptoms among volunteers, and, also, whether either agent effected immunity to reinfection and to the other virus.

One hundred fifty-nine subjects were given from four to 14 thousand infectious doses for fifty per cent of monkey kidney tissue cultures. Among 90 who received 2060, 25 per cent developed a coryzal illness; among 69 given J.H., 34 per cent developed illness. The rate of illness among 96 simultaneously uninfected subjects who served as controls was 18 per cent. Thus, the total infected group had significantly more illness than control subjects. The illness was characterized by a short incubation period. Nasal discharge and obstruction were the principal symptoms. Infection was proved by reisolation of J.H. virus from 53 per cent of one group of volunteers including one-half of those without any clinical symptoms. Volunteers given either 2060 or J.H. were significantly immune to rechallenge with the same or other viruses. Immunity to J.H. also was demonstrated by the absence of recoverable virus in nasal secretion after rechallenge.

The data show that tissue culture harvests of 2060 and J.H. viruses can produce an afebrile coryzal illness in human beings. Their infectivity is of low order or there is a high degree of immunity among volunteers. Infection occurs in the absence of symptoms. Prior challenge prevents reinfection. Among volunteers the viruses 2060 and J.H. are immunologically identical.

Effect of Gluten and of Cortisone upon Experimental Malabsorption Induced by Neomycin. EUGENE D. JACOBSON, ROBERT B. CHODOS, SUSAN HIBBS and WILLIAM W. FALOON, Syracuse, N. Y. (Introduced by Eugene L. Lozner).

Attempts to reverse the malabsorption syndrome induced by neomycin have been made, employing gluten free diets and cortisone. Parameters used included fecal fat, plasma carotene, D-xylose tolerance tests and I-131 triolein (RITO) absorption and excretion.

Four subjects were studied during periods of: *1*) constant gluten diet, *2*) same diet plus 12 Gm. neomycin daily, and *3*) same diet minus gluten foods plus neomycin. Carotene fell during Period *2*. Gluten removal (Period *3*) yielded rising carotene in two subjects and no further decline in two. Fecal fat was determined in the first two subjects. Excretion doubled during Period *2* and was unchanged with gluten removal. D-Xylose absorption in these same two subjects was normal in Period *1* and fell to half in Periods *2* and *3*.

Four subjects were studied during six to eight day periods of: *1*) control, *2*) 12 Gm. neomycin daily, and *3*) neomycin plus cortisone, 100 mg. daily orally. Carotene fell in all four during neomycin and rose again in two of these when cortisone was added. D-Xylose determined in three fell to 50 per cent of control values but was unchanged by cortisone. As a control, one subject received cortisone without neomycin and his plasma carotene increased.

Blood RITO levels and fecal RITO excretion in general paralleled the carotene and fecal fat changes during neomycin but analysis of these data as well as data from other subjects suggest that blood and fecal RITO levels are less reliable parameters of malabsorption than carotene, fecal fat and D-xylose.

The data indicate that in the malabsorption syndrome produced by neomycin the only consistent change induced by gluten exclusion or cortisone is a rise or leveling off of plasma carotene concentration. These carotene changes are independent of alterations in fecal fat and D-xylose.

Abnormal Patterns of Stercobilin Formation in Dyserythropoiesis. G. WATSON JAMES, III* and LYNN D. ABBOTT, JR., Richmond, Va.

Bile pigment metabolism in hematologic disorders was investigated by labeling nitrogen of fecal stercobilin through 1 Gm. doses of N^{15}-glycine (33 atom per cent N^{15}). Of particular interest were conditions in which elevated fecal urobilinogen was not associated with increased peripheral blood destruction. The N^{15}-stercobilin and N^{15}-hemin data indicate increased bile pigment formation independent of hemoglobin degradation and not coupled with hemoglobin synthesis.

In two normal subjects stercobilin crystallized from four day fecal periods contained 0.022 and 0.021 atom

per cent excess N^{15}. The N^{15} concentration diminished quickly in the next four day period. In striking contrast, two patients with folic acid and vitamin B_{12} unresponsive megaloblastosis, leukopenia and monocytosis had stercobilin N^{15} concentrations of 0.098 and 0.071 initially, increasing to 0.142 and 0.116 in the next period, then gradually decreasing for the remaining three four day collections. One patient with erythroid aplasia showed an initial content of 0.045, increasing to 0.070 in stercobilin, while simultaneously showing *no* uptake in the circulating heme. An adult with erythroid hypoplasia and a child with aplastic anemia demonstrated the same pattern of increasing stercobilin N^{15} with only small amounts appearing in the circulating heme. Two patients with aplasia later died with acute granulocytic leukemia. One subject with lead poisoning, coproporphyrinuria and porphobilinogenuria, studied in two day periods, demonstrated a rapidly increasing label for the first eight days. Two patients with chronic granulocytic leukemia had initially high labels of 0.063 and 0.075 atom per cent excess N^{15} with a gradual decline.

Increased incorporation of N^{15} from glycine into stercobilin suggests that altered pyrrole metabolism may occur in a wide variety of seemingly unrelated hematologic disturbances. Increased fecal urobilinogen excretion is not necessarily a result of hemolytic disease and may be "anabolic" in nature. In such patients fundamental biochemical derangement of pyrrole metabolism may exist.

Intrahepatic Conjugation of Bromsulphalein and Glutathione. NORMAN B. JAVITT, HENRY O. WHEELER, KATHERINE J. BAKER and OSWALDO RAMOS, New York, N. Y. (Introduced by Stanley E. Bradley).

Recent work by Brauer, Meltzer, Combes and associates indicates that bromsulphalein (BSP) appearing in bile of man, dog and rat during intravenous administration may be separated by paper chromatography into at least four fractions having identical absorption spectra in alkaline solution. One is free BSP and the others appear to be amino acid conjugates. The present work indicates that linkage of BSP with glutathione through the sulfhydryl group may account in large part for this phenomenon.

Paper electrophoresis (600 V., 0.5 ma. per cm., pH 1.9, 5 N acetic acid) was used to remove free amino acids and proteins (cathodal migration) from BSP and its products (anodal) in canine bile obtained by Thomas fistula.

The major conjugates were eluted, fractionated chromatographically (*n*-butanol, acetic acid, water), re-eluted and hydrolyzed (0.3 N NaOH, two hours, 80° C.). The hydrolysates always contained glutamic acid and glycine in association with evidence of free sulfhydryl groups (positive sodium azide-iodine reaction). Cysteine was occasionally detectable. Admixture of glutathione (0.3 per cent) and BSP (0.9 per cent) solutions *in vitro* (NaOH, pH 7 to 8, 25° C., two hours) resulted in the appearance of BSP conjugates identical by electrophoresis and chromatography with conjugates formed *in vivo*. Oxidation or formation of mercapturic acid derivatives may account for difficulty in detecting cysteine in hydrolysates. Following equilibration two hours after injection of S^{35} labeled glutathione (100 μc., 6.1 mg.) in one dog, the radioactivity of the bile rose (fivefold) and fell with BSP excretion.

Biliary BSP "metabolites" observed in man and animals may therefore result from intrahepatic conjugation of BSP and glutathione with the production of a variety of isomers and degradation products incidental to transfer from blood to bile. The hepatic glutathione content may be affected by and may influence BSP excretion.

Effects of Intravenous Hypertonic Saline in Patients with Hyponatremia and Edema. BEN B. JOHNSON and KATHLEEN E. ROBERTS,* San Francisco, Cal.

Treatment of hyponatremia and edema with hypertonic saline is frequently given with the intention of producing an increase in serum sodium concentration and benefit to the patient. More recently, however, the possible role of water retention with overexpansion of extracellular fluid volume has been re-emphasized in some cases of hyponatremia and edema ("the dilution syndrome"). A few reports have appeared of such cases in which serum sodium was not increased by administration of hypertonic saline. The present study was attempted to investigate whether hyponatremia does represent dilution in a substantial number of these patients and whether this treatment can regularly increase serum sodium. Patients were selected primarily because they had been treated with hypertonic saline; all patients had some degree of edema and with one exception serum sodium was below 125 mEq. per L. before treatment. The series included, among others, all such patients treated on one service during the past year. A wide range of renal, hepatic, cardiac, infectious and other diseases was represented.

On 35 occasions, 27 patients were treated with 0.15 to 2.0 L. of 2.5 to 5.0 per cent NaCl solution. Initial serum sodium ranged from 94 to 134 mEq. per L., and increased in seven patients by from 5 to 12 mEq. per L. after treatment. On 26 occasions in the remaining 20 patients, serum sodium concentration was not significantly changed or occasionally decreased even further. More than 90 per cent of the patients showed evidence of extracellular fluid expansion as indicated by increased peripheral edema, ascites or pulmonary congestion. In three such patients treatment was repeated; each patient died shortly after the second or third injection of hypertonic saline.

These studies confirm isolated reports that hypertonic saline may be ineffective in the hyponatremia of the dilution syndrome, and indicate that such treatment usually increases edema and frequently does not result in clinical improvement.

Vitamin B-12 Binding and Human Intrinsic Factor Activity. P. C. JOHNSON, Oklahoma City, Okla. (Introduced by Stewart Wolf).

Fractions of gastric content obtained from six human subjects and separated by the resin column technique of Richmond and co-workers (Arch. Biochem. 1957, 66, 155) have been studied for vitamin B-12 binding by a dialysis method for intrinsic factor activity by the rat liver slice technique of Miller and Hunter, and by cobalt-60 vitamin B-12 liver uptake in pernicious anemia patients. In the gastric content from three healthy humans, binding was identified in only three of the five protein peaks eluted from the resin column. These included the major carbohydrate peak, one of the two peaks containing all of the proteolytic activity and a third peak whose physiological properties are still unidentified. No binding was present in the others. No uptake was demonstrated by the rat liver slice technique until the five peaks were separated by chromatography; then all five showed uptake. The binding peaks as well as preparations of the whole juice demonstrated intrinsic factor activity when tested on pernicious anemia patients. The gastric content of two pernicious anemia patients showed only four protein peaks on chromatography. Three of them bound vitamin B-12 but no fraction showed proteolytic activity, uptake on the rat liver slice or intrinsic factor activity in pernicious anemia patients. On the contrary, gastric content from one nonanemic subject whose B-12 uptake was normal failed to bind vitamin B-12 and showed no proteolytic activity; yet its four protein fractions showed uptake in the rat liver slice and in a pernicious anemia patient. It would appear from these data that B-12 binding does not necessarily imply human intrinsic factor activity and human intrinsic factor activity does not necessarily involve B-12 binding.

Circulatory and Electroencephalographic Changes Associated with Loss of Consciousness in Vasodepressor Syncope. H. R. KARP, A. M. WEISSLER and A. HEYMAN,* Durham, N. C.

Recent interest in circulatory adaptation to rapid acceleration has led us to reinvestigate the changes in arterial pressure, pulse rate and the electroencephalogram (EEG) as predictive indices of loss of consciousness in posturally induced vasodepressor syncope.

Vasodepressor syncope was induced in 10 normal young male subjects by sodium nitrite (180 mg. orally) combined with 60 degree head-up tilt. Continuous EEG recordings were made simultaneously with pulse and direct arterial blood pressure determinations.

Before onset of syncope, the blood pressure showed a progressive reduction associated with autonomic dysfunction, *e.g.*, sweating, pallor and nausea. Overt disturbances of mental function were a late phenomena and coincided with the appearance of sporadic low voltage theta waves in the EEG. Loss of consciousness developed four to 10 seconds later and was accompanied by a profound reduction in mean arterial pressure to an average of 31 mm. Hg (range, 22 to 42) and by high voltage delta waves in the EEG. At this time the mean arterial pressure had fallen approximately 70 per cent from control levels and the cerebral blood flow, as estimated by cerebral arteriovenous oxygen differences, showed a 50 per cent reduction. The development of bradycardia was a late but inconsistent finding.

These studies demonstrate a remarkable adaptability of the central nervous system of normal subjects to withstand severe hypotension. The most reliable indices for predicting loss of consciousness appeared to be the appearance of theta activity in the EEG and a fall in mean arterial pressure below 45 mm. Hg.

Biochemical and Immunochemical Studies on the Low Density Lipoproteins of Human Serum and Aortic Wall. HERBERT J. KAYDEN, BEATRICE C. SEEGAL and KONRAD C. HSU, New York, N. Y. (Introduced by J. Murray Steele).

The distribution of the phospholipids of whole serum and of the alpha and beta lipoproteins (separated by starch block electrophoresis) between the glycerophosphatide and sphingomyelin fractions was measured by differential alkaline hydrolysis and by silicic acid column chromatography in 50 men and women of different ages. The phospholipid composition of alpha lipoprotein averaged 90 per cent glycerophosphatide and 10 per cent sphingomyelin; of beta lipoprotein, averaged 75 per cent glycerophosphatide and 25 per cent sphingomyelin. The serum glycerophosphatides were equally divided between the alpha and beta lipoprotein fractions, but three-quarters of the serum sphingomyelin was present in the beta fraction. The partition of serum cholesterol closely resembled the partition of serum sphingomyelin and the ratio of sphingomyelin to cholesterol was constant for both groups of lipoproteins.

Studies of the phospholipid composition of the inner aortic wall of 24 subjects revealed a marked increase in the amount and proportion of sphingomyelin to total phospholipid as aging and atherosclerosis develop. A large proportion of the lipids extractable with saline from aged and/or atherosclerotic vessels was in the low density lipoprotein fraction (specific gravity < 1.063). Sphingomyelin was the major phospholipid component in this lipoprotein fraction.

Rabbits were immunized with the low density lipoproteins obtained, under comparable conditions of ultracentrifugation, from human sera and from saline extracts of human aortas. The reactions of the antisera with low density lipoproteins of serum and aorta were studied by means of the precipitin test, by double diffusion patterns in gels (Ouchterlony) and by immunoelectrophoresis (Grabar). These tests failed to distinguish between the two antigens. Thus, although the serum and aortic wall lipoproteins showed marked differences in lipid composition, no differences in the immunochemical nature of the protein moiety of these lipoproteins could be demonstrated.

Dosage-Response Studies during the Resistant Phase in Primaquine-Sensitive Hemolytic Anemia. ROBERT W. KELLERMEYER, ALVIN R. TARLOV, STANLEY L. SCHRIER and PAUL E. CARSON, Chicago, Ill. (Introduced by Alf S. Alving).

Clinical hematological recovery from acute hemolysis induced by daily administration of a "standard test dose" of primaquine (30 mg. base) occurs despite its continued administration; nevertheless, the biochemical and enzymatic abnormalities of the erythrocytes persist. However, alterations in abnormalities in the primaquine-sensitive erythrocytes, some of them toward "normal," can be demonstrated during the resistant phase after hemolysis. Three to four months after initiating daily administration of 60 mg. primaquine to four sensitive males, the following changes in their erythrocytes from pretreatment values were found: 40 per cent reduction of the Cr^{51} half-life; detectable increase in reticulocytes; 65 per cent increase in glucose-6-phosphate dehydrogenase activity; 30 per cent reduction of catalase activity from pretreatment low values which have recently been described to be characteristic of this disorder.

Beutler and co-workers have shown that erythrocytes of primaquine-sensitive individuals are resistant to 30 mg. primaquine during the cell age period eight to 21 days, and are sensitive during the cell age period 63 to 76 days. By employing increasing doses at various times after hemolysis, we have demonstrated that the resistance of the younger erythrocytes is not absolute but is relative both to cell age and to drug dosage. Increasing the daily dose in one volunteer to 120 mg., one week after he had experienced acute hemolysis on 30 mg., precipitated a second major hemolysis during subsiding reticulocytosis; in a second individual after the ingestion of 30 mg. for more than one year, an increase in the dose to 240 mg. resulted in another acute hemolysis. Similar results have been obtained with studies carried out at other dosages and times in several volunteers after the initial hemolytic episode.

It is concluded that erythrocytes of all ages in primaquine-sensitive individuals are probably susceptible to this type of chemical hemolysis. Primaquine-sensitive erythrocytes become progressively vulnerable with age to drug induced hemolysis.

Energy Expenditure and Utilization of Carbohydrate, Fat and Protein in Hospitalized Patients. JOHN KINNEY, HARVEY ZAREM and RICHARD ROGERS, Boston, Mass. (Introduced by Francis D. Moore).

The twenty-four hour values for urinary nitrogen excretion, oxygen consumption and carbon dioxide production allow determination of the total caloric expenditure and the amounts of carbohydrate, fat and protein catabolized by an individual. Application of this technique of indirect calorimetry, established by Atwater and Benedict, has been perfected for use on a metabolic ward. Ten to 14 five minute samples of expired air, taken at regular intervals throughout each day, are analyzed to provide measurements of gas exchange. Conventional nitrogen balance techniques and known food intake supply additional information for calculating total energy balance and individual balances of carbohydrate, fat and protein.

Fifteen patients and one control subject were studied for periods of eight to 21 days. Alimentation included conventional foods, purified synthetic diets, intravenous carbohydrate, protein hydrolysates and alcohol. Energy balance was determined under conditions of light activity, semistarvation, rapidly progressing carcinoma and trauma imposed by surgery of varying magnitude. Actual changes in body weight were compared with theoretical weight changes calculated from the cumulative balance of fat and lean tissue.

These studies indicate that during periods of negative calorie balance, oxidation of carbohydrate is reduced despite intake of a mixed diet. After surgical trauma, with caloric intake limited to intravenous carbohydrate, patients oxidize decreased amounts of carbohydrate until a mixed diet approaching caloric balance is achieved. Possible explanations for this finding are discussed in the light of currently accepted intermediary pathways.

The trauma of elective surgery does not increase total caloric expenditure although, postoperatively, the basal expenditure increases approximately 10 per cent over preoperative levels for four to seven days. Weight loss of simple starvation is associated with decreased caloric expenditure; however, studies of two patients with progressive weight loss from inoperable gastric carcinoma revealed an elevated caloric expenditure which slowly increased without fever in the late weeks of the disease.

The Regulation of Glucose Uptake by Muscle: Significance of Cellular Permeability and Hexokinase Activity. DAVID M. KIPNIS, St. Louis, Mo. (Introduced by Carl V. Moore).

The functional significance of cellular permeability (sugar transport) and hexokinase activity in the regulation of glucose uptake by muscle and their response to a variety of hormones known to influence muscle glucose metabolism have been examined with the following techniques: *1)* The rates of sugar transport were measured in an "intact" diaphragm preparation with 2-deoxyglucose as described in previous studies from this laboratory. *2)* Hexokinase activity was measured as the rate of 2-deoxyglucose phosphorylation by the usual "excised" diaphragm preparation. *3)* The relative rates of penetration and phosphorylation were estimated by determining the distribution of glucose in the "intact" diaphragm; intracellular glucose accumulated if penetration exceeded phosphorylation but remained extracellular if penetration was rate-limiting.

Sugar transport was rate-limiting in the utilization of glucose by normal nonfasted muscle over a wide range of sugar concentrations (0.005 M to 0.08 M).

Cellular permeability was not affected by epinephrine. Hexokinase activity, however, was markedly inhibited by the high levels ($> 2 \times 10^{-3}$ M) of glucose-6-phosphate, a noncompetitive inhibitor of hexokinase ($K_i - 4 \times 10^{-4}$ M), which accumulated intracellularly as a result of the increased rate of glycogenolysis.

Insulin lack (alloxan diabetes) resulted in a marked impairment of cellular permeability ($\sim$ 90 per cent) and a moderate inhibition of phosphorylation ($\sim$ 50 per cent). Adrenalectomy did not alter the severely depressed rate

of sugar transport, but reversed the inhibition of phosphorylation.

Although hexokinase activity was increased (~50 per cent) following hypophysectomy, a diminished rate of sugar transport was observed which was interpreted as reflecting the decreased levels of insulin in the hypophysectomized animal.

These studies indicate that glucose utilization may be limited by either sugar transport or hexokinase activity. Although epinephrine, adrenal glucocorticoids and growth hormone influence hexokinase activity, their effects will not be observed when sugar transport is rate-limiting (*i.e.*, insulin deficiency) as in the diabetic or fasting hypophysectomized animal.

The Combined Influence of the Adrenal and Parathyroid Glands in the Regulation of Serum Calcium. CHARLES R. KLEEMAN,* DONALD BERNSTEIN, J. THOMAS DOWLING, JERRY M. KOPLOWITZ and MORTON H. MAXWELL, Los Angeles, Cal.

Adrenal steroids may significantly lower the serum calcium in many hypercalcemic disorders and aggravate the hypocalcemia of hypoparathyroidism. Hypercalcemia may occur in acute adrenal insufficiency. The mechanism of these effects on serum calcium is unknown, but it is not due to the effect of adrenal steroids on calcium balance (Meyers and co-workers, J. Clin. Invest. 1958, 37, 919).

The interaction of the parathyroids and adrenals on calcium mobilization was investigated in acute experiments on calcium depleted rats. Adrenalectomy was performed two days *prior to* parathyroidectomy; animals drank saline *ad libitum*. Controls were sham operated. Values represent the means six hours after parathyroidectomy.

Parathyroidectomy caused a marked decrease in serum calcium (9.2 to 5.3 mg. per cent), a slight rise in serum phosphorus (7.2 to 8.0 mg. per cent) and profound decrease in the six hour phosphorus excretion (3.4 to 0.8 mg. per 100 Gm. rat). Prior adrenalectomy significantly inhibited postparathyroidectomy hypocalcemia (6.9 mg. per cent compared to 5.3 mg. per cent) *without* alteration of serum phosphorus or hypophosphaturia. The calcium mobilizing effect of 80 units of parathyroid extract (PTE) given at zero time to sham and parathyroidectomized animals was significantly enhanced by adrenalectomy (10.3 and 9.1 mg. per cent, respectively). The phosphaturic effect of PTE *was not* enhanced. Adrenalectomy *per se* did not cause hypercalcemia.

Parathyroidectomy caused a marked *decrease* in serum citrate (11.4 to 5.1 mg. per cent) and a marked *increase* in citrate excretion (50 to 530 μg. per 100 Gm. rat). Adrenalectomy *prevented* the fall in serum citrate and the rise in urinary citrate following parathyroidectomy.

Adrenalectomy + parathyroidectomy + 80 units PTE significantly *enhanced* serum citrate (15 mg. per cent) and *decreased* urinary citrate (30 μg. per 100 Gm. rat). Studies are in progress to measure tissue and bone citrate and the effect of exogenous adrenal steroids.

The adrenal cortex may exert a "tonic" suppressive effect on the calcium mobilizing mechanism(s) of bone. Hypoparathyroid hypocalcemia was significantly inhibited by adrenalectomy and the latter significantly enhanced the response to parathyroid extract. These changes may be due to the interrelationship of the adrenal and parathyroid glands on citrate metabolism.

Reproduction of the Historical Sequence of Development of Resistant Staphylococci in Hospitals. VERNON KNIGHT,* THOMAS HEMMERLY, MARGARET P. MARTIN and ARTHUR WHITE, Nashville, Tenn.

In a one year study, selected carriers and noncarriers of staphylococci among 50 mentally ill patients have been given treatment in sequence with penicillin, tetracycline, erythromycin, oleandomycin, penicillin and tetracycline or erythromycin. The first studies with penicillin and tetracycline have been previously reported. They showed a shift from a high predominance of Phage Group I strains susceptible to common antibiotics, to a predominance of strains resistant to penicillin or penicillin and tetracycline, about equally divided among Phage Group II, III or no type. Following erythromycin, resistance to this agent was superimposed on resistance to the other drugs, and the cultures shifted to more than 70 per cent Phage Group III. This distribution has been maintained throughout other drug-treatment experiments.

This study has, therefore, reproduced within one year, under carefully controlled conditions, the events which have occurred in numerous hospitals throughout the world in the last 15 years. It is of interest that the shift to Phage Group III occurred following administration of the third drug, erythromycin. This appears to be in accord with the history of staphylococcal resistance except that it followed a different sequence of drugs. Observations are now continuing to detect the predicted appearance of Phage Type 80/81 strains to complete this experimental analogy with historical events.

Studies on the Pathogenesis of Anemia with an Iron Chelate. SAMUEL KORMAN, New York, N. Y. (Introduced by Robert G. Bloch).

The intravenous administration of radioiron (containing 0.6 mg. carrier iron) chelated with ethylenediamine di-*o*-hydroxyphenylacetic acid (EDDHA) permits the differentiation of anemia due to iron deficiency, infection, renal disease and decreased erythropoiesis. The half-time of the plasma Fe^{59} removal, the urinary excretion of the iron chelate (Fe^{59} and total Fe) and the erythrocyte uptake of the retained radioiron were determined. The half-time of the plasma Fe^{59} removal for normal subjects ranged from one to three hours. Twenty to 35 per cent of the administered Fe^{59} was excreted in the urine within four hours. The excretion of the administered total iron was greater than that of Fe^{59}. This dilution of the specific activity represents the exchange of the injected Fe^{59} with the labile iron of the iron stores.

Iron deficient patients had an accelerated removal of the injected Fe^{59} from the plasma. The urinary Fe^{59}

excretion was decreased with the total iron excretion remaining the same as the Fe^{59} excretion. This indicates that these patients have decreased labile iron and that they have a greater affinity for the chelated iron than normal subjects.

The Fe^{59} excretion of patients with anemia of infection was in the normal range and the total iron excretion was very similar to the Fe^{59} excretion. The normal Fe^{59} excretion indicates normal iron stores, whereas the slight decrease of the specific activity indicates diminished labile iron. The erythrocyte uptake of the retained Fe^{59} was in the normal range.

Patients with renal insufficiency had a decreased Fe^{59} excretion, with the total iron excretion much greater than the Fe^{59}. The decreased specific activity of the excreted Fe^{59} indicates normal labile iron. The erythrocyte uptake of the retained Fe^{59} was decreased in one-third of these patients, indicating decreased erythropoiesis.

The use of this radioiron chelate permits, for the first time, the evaluation of the iron stores in addition to an estimation of the erythropoietic activity, in one single study.

Effect of 2-Substituted Thiadiazoles on Uric Acid Synthesis in Man. IRWIN H. KRAKOFF and M. EARL BALIS, New York, N. Y. (Introduced by David A. Karnofsky).

2-Ethylamino-1,3,4-thiadiazole and the 2-amino- and 2-acetylamino-analogues have been found to cause an increase in *de novo* synthesis of uric acid in man. This has been demonstrated by several means. A parallel increase in uric acid content in blood and urine occurred during the administration of these compounds. Prompt incorporation of sodium formate-C^{14} and ammonia-N^{15} into uric acid was increased during thiadiazole administration as compared with control studies in the same subjects. The concentration in the urine of uric acid-C^{14} derived from previously administered adenine-C^{14} dropped sharply during thiadiazole administration, indicating dilution with newly synthesized, unlabeled uric acid. Clinical observations and measurement of nitrogen excretion confirmed that tissue breakdown did not contribute to the increased uric acid production.

Simultaneous administration of nicotinamide in doses equal to or greater than the dosage of thiadiazole modified or completely blocked the uricogenic effect and the characteristic glossitis produced by the thiadiazoles. The glutamine antagonists, 6-diazo-5-oxo-L-norleucine (DON) and *o*-diazo-acetyl-L-serine (azaserine), which are known to inhibit purine biosynthesis in several animal systems, blocked both the uricogenic effect of the thiadiazoles and the increased incorporation of formate-C^{14} into uric acid without modifying the glossitis.

The related 2,5-substituted compounds, 2,5-diamino-1,3,4-thiadiazole and 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (acetazolamide, Diamox®) produced neither the uricogenic effect nor the glossitis, indicating a close relationship of activity to structure.

The mechanism of the uricogenic effect of the thiadiazoles has not been determined. It is postulated that they block the incorporation of newly synthesized purine molecules into coenzymes and/or purine polynucleotides, with a resultant compensatory exaggeration of *de novo* purine biosynthesis leading to increased uric acid production. The reversal by nicotinamide suggests a relationship to the nicotinamide-containing coenzymes which has not yet, however, been defined.

The Effect of Diet upon the Diurnal Serum Triglycerides of Patients Having Hyperlipidemia, Cystic Fibrosis of the Pancreas and of Healthy Subjects. PETER T. KUO and JOHN C. CARSON, Philadelphia, Pa. (Introduced by Calvin F. Kay).

An elevated serum triglyceride level has been correlated with coronary disease, and with certain undesirable effects upon capillary circulation and blood coagulability. However, observations have been limited to the fasting levels of this labile serum lipid fraction. In this study, the diurnal variations in serum triglycerides of nine healthy and diseased subjects, stabilized sequentially on isocaloric regular, rice and 50 to 70 per cent corn oil formula diets, were determined. Fat absorption in nine children with cystic fibrosis of the pancreas was controlled by pancreatic extract. Diurnal serum lipid variations of these children on diets supplemented with predigested proteins and carbohydrates were studied before and after pancreatic extract (Viokase®) administration. In all subjects, serum phospholipid and cholesterol were also measured; their diurnal variations were minor. The postprandial serum triglyceride elevations were lower and briefer while on corn oil diet than on regular diet in four healthy subjects and one with hypercholesteremia. This difference in effects of unsaturated from saturated dietary fats was progressively less evident in four patients with increasingly severe hyperlipemia. In nonlipemics on rice diets, fasting serum triglycerides were only slightly higher than while on regular and corn oil diets, but the mean diurnal levels were lower. In lipemics on rice diets, varying degrees of fasting hyperlipemia were observed. Diurnal serum triglyceride curves of nine children with cystic fibrosis of the pancreas were similar to those of healthy adults on rice diets. The mean fasting serum triglyceride level of the diseased children was the same as that of the seven controls. After Viokase® administration, significant postprandial rises in serum triglycerides were observed in six of them. The data suggest that on a low fat diet hyperlipemics tend to mobilize endogenous triglycerides excessively during fasting. Correlation of this phenomenon with the development of atherosclerosis is being investigated.

Some Actions of Neurohypophyseal Hormones on a Living Membrane. EZRA LAMDIN, ROY H. MAFFLY, RICHARD M. HAYS and ALEXANDER LEAF,* Boston, Mass.

The isolated toad bladder, essentially a single layer of cells, transports sodium actively from its mucosal to

serosal surface. This transport is markedly stimulated by both oxytocin and vasopressin. The increased sodium transport resulting from hormonal stimulation is associated with an increased rate of energy metabolism; oxygen uptake is increased and glycolysis accelerated. The stimulation of sodium transport by hormone also occurs anaerobically. The sodium movement in the opposite direction and the diffusion of other ions through the membrane are unaffected by hormone. Only the diffusion of urea was found to be increased regularly (*circa* 10-fold) in both directions by hormone. Water flux in the absence of an osmotic gradient is little or not at all affected but in the presence of an osmotic gradient water flux is definitely increased. The action on the passive flux of urea is not dependent upon the presence of sodium in the medium and takes place in the absence of a demonstrable effect on energy metabolism. In the case of urea the action of the hormone is shown to be associated at least in part with an increased permeability through a diffusion barrier at the mucosal surface of the cells. The effect of the hormone in increasing the osmotic flow of water through the membrane is consistent with the pore theory of action but the specific effect only on permeability to urea and on the active sodium transport suggests a more complex action of the hormone than simply to dilate aqueous channels through the membrane.

Multiple Site Indicator Dilution Curves in Dogs With and Without Metered Left Heart Regurgitation. RAMON LANGE and HIROSHI KUIDA, Salt Lake City, Utah. (Introduced by Hans Hecht).

A method for quantitation of valvular regurgitation in man from simultaneously recorded pulmonary artery (PA) and femoral artery (FA) indicator dilution curves following venous injection was based on the extreme similarity of these curves in the absence of regurgitation. This led to the use of the deviation of the FA curve from the PA (control) curve as a measure of the regurgitation fraction of left ventricular stroke volume. Assuming that left heart regurgitation prevents the near identity of all transit times found in the nonregurgitant system, it would then cause the average transit time between sites to exceed the shortest transit time. These values from FA and PA curves allow calculation of the regurgitant to net flow ratio Q_R/Q_F. This method agreed with internal checks and surgical findings, but since independent measurement is unavailable in man, animal studies were done.

Simultaneous curves were recorded from the PA and FA following venous injection in 12 anesthetized dogs: *a*) in closed chest, *b*) in open chest, *c*) with left atrial (LA) transfusion of undyed blood, and *d*) with surgically induced aortic-LA "regurgitation" via an orifice meter.

When precautions were taken to avoid certain sampling errors, the similarity of PA and FA curves was as striking as in humans. The open chest preparation without regurgitation caused variable skewing of the FA curve whch could be minimized by suspension of the unsupported mediastinal structures or by LA transfusion of undyed blood. With metered regurgitation (Q_R) from 20 to 70 per cent of the net forward flow (Q_F) as determined by dye output, agreement occurred between derived and measured values with a correlation coefficient of 0.88. These results are interpreted to support the method previously reported in humans.

The Binding of Myoglobin by Plasma Protein. WILLOUGH LATHEM,* Pittsburgh, Pa.

When extracorpuscular hemoglobin is added to plasma, an interaction occurs with plasma proteins and hemoglobin becomes bound. The present study was undertaken in order to determine whether a similar interaction occurs between myoglobin and plasma protein.

Myoglobin was added to dog plasma *in vitro* and *in vivo* in concentrations of 5 to 150 mg. per cent. The electrophoretic characteristics of myoglobin in plasma were then determined by paper electrophoresis, utilizing benzidine-hydrogen peroxide for staining purposes.

At pH 7.0 and 8.6 myoglobin was separated into two fractions, one of which had the characteristics of free, unbound myoglobin and of free hemoglobin. This fraction appeared in the urine. The second fraction, which migrated between alpha-2 and beta globulin, resembled protein-bound hemoglobin electrophoretically and was not excreted. The concentration of myoglobin in this fraction was limited, maximally, to 24 ± 6 mg. per cent.

These observations indicate that myoglobin exists in plasma in the free and protein-bound states and that the binding capacity is limited. At concentrations below the binding capacity from 15 to 50 per cent of the myoglobin was in the free state, differing from hemoglobin which was completely bound below the hemoglobin binding capacity (123 mg. per cent).

When myoglobin was added to plasma containing bound hemoglobin, the binding of myoglobin was blocked. At low concentrations the binding of hemoglobin was not altered by the presence of bound myoglobin. However, at high concentrations the total (myoglobin plus hemoglobin) bound material did not exceed the hemoglobin binding capacity. These observations indicate that myoglobin and hemoglobin share and/or compete for at least some of the same binding sites.

The demonstration of myoglobin binding and of the excretion of free but not protein-bound myoglobin indicates that such binding determines or contributes to the apparent renal threshold to myoglobin.

The Influence of Sympathetic and Vagal Stimulation on the Relationship Between Left Ventricular End-Diastolic Pressure and Fiber Length. RONALD J. LINDEN, JERE MITCHELL and STANLEY J. SARNOFF,* Bethesda, Md.

The performance characteristics of the heart have been expressed in terms of the relationship between filling pressure and the work performed largely because the more fundamental parameter, that of fiber length, has been difficult to measure. An instrument has been de-

signed to record the changes in distance between two points in the left ventricular myocardium. With this instrument the changes in the end-diastolic length of a segment of ventricular muscle over a wide range of end-diastolic pressures, *i.e.*, a ventricular end-diastolic pressure/"fiber length" curve, have been measured. Using such curves it was shown that neither stimulation of the sympathetic cardiac nerve nor stimulation of the vagal nerve produced, *per se*, any detectable change in this ventricular end-diastolic relationship. These data suggest that the ventricular end-diastolic pressure-volume relationship remains remarkably constant under autonomic nerve stimulation. Thus it may be concluded that, at a given filling pressure, the greatly augmented stroke work produced by cardiac sympathetic nerve stimulation occurs from the same end-diastolic fiber length. Finally, the fact that there are no changes in distensibility with stimulation of autonomic nerves to the heart suggests that there is a family of fiber length-stroke work curves similar to the family of filling pressure-stroke work curves and the shift from one curve to another may be accomplished over physiologically operative neuronal pathways.

Renal Mechanisms in the Production of Hypercalcemia in Hyperparathyroidism and Breast Cancer. H. F. Loken and G. S. Gordan,* San Francisco, Cal.

The nonprotein-bound fraction of total serum calcium (NPBCa) was separated from the protein-bound moiety by ultracentrifugation. By this technique, 50 to 56 per cent of total serum calcium is not protein-bound at 37° C. and pH 7.35. Other variables affecting protein binding of calcium are total ionic content and serum protein concentration, but not CO_2 content or total calcium level. Glomerular filtration rate was estimated by endogenous creatinine clearance and net tubular reabsorption from the difference between the calculated calcium load and directly measured urinary excretion. Normally the filtered load is 165 to 325 mEq. per day, of which 95 to 99 per cent is reabsorbed by the tubules so that 1.4 to 3.9 ml. per minute are cleared.

In 16 cases of primary hyperparathyroidism, NPBCa was elevated proportionate to total serum Ca, but within the normal 50 to 56 per cent distribution. The filtered load was increased except where uremia was present. Urinary excretion was increased proportionately so that calcium clearance was normal. Calculated tubular reabsorption of calcium (TRCa) was increased parallel to the filtered load. These findings contrast with those of hypoparathyroidism (12 cases) where the filtered load is decreased, clearance is subnormal and net TRCa is increased. The hypercalcemia of advanced breast cancer (nine cases) is characterized by an increased filtered load, hypercalcuria, normal or decreased TRCa and normal tubular reabsorption of phosphate. This type of hypercalcemia is often complicated by uremia, whereupon the filtered load is decreased. Clearance declines or remains normal, depending on whether TRCa is impaired. Two patients who had both hyperparathyroidism and breast cancer without uremia showed the renal phenomena of hyperparathyroidism.

Hemodynamic Observations on the Hepatic Circulation: Modifications Produced by Portacaval Shunting. Robert T. L. Long and Carlos R. Lombardo, Bethesda, Md. (Introduced by Jack Orloff).

Conflicting reports have appeared on the extent of the relief of hepatic sinusoidal hypertension following portacaval shunting procedures. This study was undertaken to investigate the relationship between hepatic wedge and portal venous pressures, and the effect on hepatic hemodynamics of end-to-side and side-to-side portacaval shunting procedures. In 16 normal dogs flows through the hepatic artery and hepatic veins were measured directly, together with hepatic arterial, vena caval and portal venous pressures. In four dogs hepatic wedge pressures were also recorded. The hepatic venous resistance averaged 488 dyne-sec-cm.$^{-5}$ per 100 Gm. of liver; the portal venous resistance averaged only 32 dyne-sec-cm.$^{-5}$ per 100 Gm. of liver. The presinusoidal hepatic arterial resistance averaged 12,500 dyne-sec-cm.$^{-5}$ per 100 Gm. of liver. Artificial portacaval shunts were created and the blood flow through these shunts was measured directly. Although creation of an end-to-side portacaval anastomosis produced a decline in hepatic wedge pressure, this decline was significantly greater after creation of a side-to-side shunt.

Flow measurements demonstrated that an average of 70 per cent of the hepatic arterial blood will actually flow through a side-to-side shunt rather than through the hepatic veins. The reduction of hepatic wedge pressure with an end-to-side portacaval shunt is simply related to the diversion of portal blood from its normal channels. The greater reduction in hepatic wedge pressure and hepatic blood flow which occurs with a side-to-side shunt results from the additional diversion of a portion of the hepatic arterial blood through the shunt. These observations would seem to be of importance in the rational choice of an operation for the relief of hepatic sinusoidal hypertension as occurs in patients with ascites and esophageal varices.

Biochemical Studies and Specific Therapy in Hepatic Glycogen Storage Disease. C. U. Lowe,* J. E. Sokal, B. H. Doray and E. J. Sarcione, Buffalo, N. Y.

Two hours after administration of a tracer dose of C-14-glucose, a liver biopsy specimen was obtained in a case of classic hepatic glycogen storage disease. Liver slices were incubated for two hours in control medium and with glucagon, epinephrine, other glycogenolytic agents and rat liver homogenate. Only in the glucagon and liver homogenate flasks was any reduction of the original glycogen level (8 per cent) observed. These findings suggest that the generally accepted theory of identity of the glycogenolytic mechanisms of glucagon and epinephrine may be incorrect.

After the *in vitro* demonstration of a glucagon effect, the patient was given intensive glucagon therapy. A

striking decrease in liver size occurred. When glucagon was discontinued, the liver enlarged. Glucagon injection during fasting hypoglycemia did not raise blood glucose, but induced a sharp rise in lactate. Glucagon injection immediately after feedings resulted in greater increases in blood glucose and delayed postprandial hypoglycemia, as compared to control feedings without glucagon. We conclude that glucagon inhibited postprandial glycogen deposition and that it stimulated glycogen breakdown to metabolites other than glucose, one of which is lactate.

The characteristic pronounced hyperlactacemia, regularly observed after minimal fasting, responded to hyperglycemia induced by glucose infusion and equally well to glucose infusion with concomitant administration of insulin, which maintained hypoglycemia. Thus the hyperlactacemia is not caused by hypoglycemia *per se*, and its amelioration is probably related to acceleration of lactate utilization (possibly in fat tissues) by exogenous or endogenous insulin.

A surprising finding was the rapid incorporation of glucose-C-14 into liver glycogen.

Since glucagon induced glycogen breakdown and inhibited glycogen deposition, a long term therapeutic regimen, based on glucagon injection in conjunction with feedings, was instituted. The child has now been under treatment for over a year and is doing well.

Antibodies in Human and Rabbit Sera to Hog Intrinsic Factor Preparations. LOUIS LOWENSTEIN, BERNARD A. COOPER, LAUDER BRUNTON and SUSAN GARTHA, Montreal, Canada. (Introduced by Ronald V. Christie).

It has been observed that certain patients with pernicious anemia become refractory to the action of orally administered intrinsic factor preparations of hog origin.

In order to test the hypothesis that antibody formation might play a role in the development of such a refractory state, antibody formation was stimulated in rabbits in response to intravenous injections of partially purified hog intrinsic factor preparations. Antibodies were demonstrated by agglutination of erythrocytes to which hog intrinsic factor had been bound by bisdiazobenzidine. After multiple absorptions with hog preparations, such as red cells and muscle extract, a residual antibody titer remained which was neutralized only by hog intrinsic factor preparations.

These antisera did not cross react with normal human gastric juice *in vitro*. When the antisera were fed to nonrefractory pernicious anemia patients together with hog intrinsic factor, the absorption of vitamin B_{12} was inhibited, as measured by the Schilling assay. Normal rabbit serum did not have this effect. When electrophoresis of normal rabbit serum preincubated with radioactive vitamin B_{12} bound to hog intrinsic factor was carried out, the major peak of the radioactivity migrated with the beta globulins. When radioactive vitamin B_{12} bound to hog intrinsic factor was preincubated with rabbit antiserum the major peak of radioactivity was associated with the gamma globulin.

Using these techniques, antibodies were demonstrated in the sera of some pernicious anemia patients refractory to hog intrinsic factor preparations. These antibodies could not be neutralized with hog muscle extract, but were neutralized by hog intrinsic factor preparations.

These studies suggest that antibodies to hog intrinsic factor are present in the rabbit antisera and in the sera of these patients. The possible role of such antibodies in the development of the refractory state in man, will be discussed.

The Uptake of Fatty Acids by Mitochondria and Adipose Tissue. W. S. LYNN, JR. and R. H. BROWN, Durham, N. C. (Introduced by Frank L. Engel).

Long chain fatty acids appear to be the major metabolic food of many cells; however, in contrast to amino acids and sugars, little information is available on the uptake by cells of fats. Nor are the relationships between various surface active agents, such as steroids, polypeptides, catechol amines or other lipids, upon the cellular transport of fatty acids understood. To explore these relationships, experiments designed to measure the exchange of various fatty acid between serum and adipose tissue or mitochondria have been done. A typical experiment was performed by incubating about 200 mg. of minced adipose tissue or mitochondria with 1 ml. of serum containing 1 μMole of a radioactive fatty acid. Serum and tissue were separated by either centrifugation or by pipetting off the serum. The lipids in both fractions were then isolated by differential solvent extractions and separated by silicic acid chromatography. Essentially all of the added fatty acid could be recovered unchanged. Dissolution and solubilization of adipose tissue were checked by weighing the lipid remaining in the washed adipose tissue at the beginning and end of the experiments. Factors which greatly facilitate this uptake of fatty acids by adipose tissues are: *1)* "aging" in the frozen state (this "aging" process can be reversed by addition of reducing substances, such as glutathione and sodium borohydride); *2)* addition of mono and diglycerides, iodoacetate, parachloromercuribenzoate and protamine, and dilution of the serum. Cyanide and triglycerides were without effect. Substances which can inhibit the uptake include oxidizable substrates, digitonin, cholesterol, cortisone, epinephrine, heparin, albumin, insulin and adrenocorticotropic hormone (ACTH). Since the above effects are highly sensitive to changes in isotonicity, concentration of such ions as K^+, Na^+ and $PO_4^=$ and the physical state of the adipose tissue, it is hoped that this system will serve as a physiological model to explore the complexities of biological surface phenomena.

The Hemagglutination Test in Hepatic Diseases. ROBERT W. MCCOLLUM and PETER ISACSON, New Haven, Conn. (Introduced by John R. Paul).

This report is concerned with an enquiry of basic mechanisms in the pathogenesis of viral hepatitis. To this end the chick erythrocyte agglutination test devised by Havens for the diagnosis of certain types of liver dam-

age, particularly that which occurs in *viral hepatitis,* has been studied. The Havens test has proved more satisfactory than other hemagglutination tests previously suggested for this and related purposes. Results based on tests of over 500 sera from patients with viral hepatitis, other hepatic diseases, nonhepatic diseases and normals indicate that, although this test is not completely specific for viral hepatitis: *1*) it may be of considerable value in differentiating between viral hepatitis and other examples of hepatic diseases with or without jaundice, and *2*) it may prove to be an indicator of the status of compensation in patients with cirrhosis, for it has been frequently found positive in advanced cases of this condition. The agglutinin which does not appear to be related to the hepatitis virus *per se* may originate from some component of the hepatic cell though its presence does not correlate directly with any of the measures of hepato-cellular damage now in common use. Questions concerned with the nature of the hemagglutination factor have been studied and are discussed.

Circulatory Dynamics in Polyostotic Fibrous Dysplasia (Albright's Syndrome). HENRY D. MCINTOSH, WILLIAM L. GLEASON, D. EDMOND MILLER and JAMES M. BACOS, Durham, N. C. (Introduced by Julian M. Ruffin).

In osteitis deformans (Paget's disease) blood flow to regions of skeletal involvement may be increased; however, the cardiac index (C.I.) is significantly increased only in very active disease. This study suggests similar circulatory alterations may accompany polyostotic fibrous dysplasia (PFD).

Five female patients with PFD were studied by accepted cardiac catheterization techniques. The catheter was also passed into the iliac, renal, and occasionally the hepatic and jugular veins to obtain regional arteriovenous oxygen differences ($A\text{-}VO_2$). Other causes for increased blood flow were excluded by appropriate studies.

Two patients (aged six and 31 years) exhibited precocious puberty, pigmentation, extensive skeletal involvement and alkaline phosphatase values of 59.3 and 9 Bodanksy units. In both, the iliac $A\text{-}VO_2$ was less than 1.7 volumes per cent; the C.I.'s were 5.6 and 6.6 L. per minute per $M.^2$, respectively. In the older patient extensive cranial involvement was associated with a continuous bruit over this area, bilateral jugular $A\text{-}VO_2$ of 2.3 volumes per cent and cardiomegaly.

One patient (aged 20) exhibiting classical pigmentation had no elevation of alkaline phosphatase and lacked evidence of precocious puberty, but did show diminution in $A\text{-}VO_2$ across involved areas as well as an increased resting C.I. (4.6 L. per minute per $M.^2$).

The two remaining patients (aged 43 and 48) showed only typical bony lesions of PFD with normal alkaline phosphatase values. The younger patient gave a history of precocious puberty; the older had classical pigmentation. Though the resting C.I.'s were not elevated, definite increases in blood flow across regions of significant bony involvement were present.

The data indicate that as in osteitis deformans, the skeletal involvement of polyostotic fibrous dysplasia may be associated with A-V shunting, and if extensive enough, local bruits, high cardiac output and cardiomegaly may occur. These data also suggest that the magnitude of the circulatory alteration may be related to the degree of activity of the osseous process as estimated by the elevation of alkaline phosphatase, and also that activity may be ameliorated with age.

Effect of the Slow Infusion of Glucagon-Free Insulin on the Hepatic Output of Glucose in Dogs with Portacaval Shunts. LEONARD L. MADISON, BURTON COMBES, WILLIAM STRICKLAND and REUBEN ADAMS, Dallas, Texas. (Introduced by Elias Strauss).

Whether or not insulin directly effects hepatic glucose metabolism has been a controversial issue. An hepatic effect is suggested both by derangements in hepatic glucose and fat metabolism characteristic of diabetes mellitus and by the fact that endogenous insulin reaches the liver in amounts and concentrations greater than that possible for any other organ. Nevertheless, *in vitro* effects of insulin on hepatic tissue have not been unequivocally demonstrated, nor have *in vivo* changes in *hepatic* glucose output been shown by direct measurement.

These experiments were designed to determine the effect of insulin infusion in dogs with complete end-to-side portacaval shunts. In this preparation only arterial blood perfuses the liver, thereby permitting measurement of changes in *hepatic* rather than *splanchnic* glucose output.

In 14 experiments of 70 to 120 minutes' duration, hepatic blood flow was measured by the technique of Bradley using radioactive rose bengal. Simultaneous arterial and hepatic-venous blood specimens were collected at 10 minute intervals during the 30 minute control period and during insulin infusion (0.1 to 0.2 U per Kg. per hour). Blood glucose was determined in triplicate by the Somogyi iodometric method. Position of the hepatic-venous catheter was checked by fluoroscopy throughout each experiment.

Results indicated a profound effect of insulin upon hepatic glucose output. During the first hour, mean hepatic venous-arterial glucose difference decreased significantly ($p = < 0.01$) from the control value of 17.7 to 8.8 mg. per cent. Concomitantly mean hepatic glucose output fell from 42 to 24 mg. per minute. The decrease in mean arterial blood glucose concentrations from 79 to 59 mg. per cent could be accounted for entirely by the reduction in hepatic glucose output.

These data show not only a significant direct effect of insulin on hepatic glucose output in the intact dog but also suggest that the liver responds to lower levels of circulating insulin than do the peripheral tissues.

Further Characterization of the Enzymatic Defect in Erythrocyte Glucose-6-Phosphate Dehydrogenase Deficiency: A Genetically Determined Trait. PAUL A. MARKS * and RUTH T. GROSS, New York, N. Y. and San Francisco, Cal.

Genetically determined enzymatic deficiency may result from different types of disturbances, including: *1*) suppression of enzyme synthesis, or *2*) formation of an abnormal enzyme.

This first possibility was examined by determining glucose-6-phosphate dehydrogenase (G-6-P.D.) in leukocytes and liver of subjects with markedly deficient erythrocyte enzyme (associated with susceptibility to hemolytic anemia following ingestion of certain agents). G-6-P.D. in leukocytes of 14 subjects with erythrocyte deficiency and 31 control subjects did not differ significantly, averaging 39.8 and 46.0 units, respectively. In three persons with deficient erythrocyte G-6-P.D., hepatic activities were 0.04, 0.05 and 0.03 unit compared with 0.05 to 0.13 unit in eight control hepatic biopsies. Thus, the abnormal gene does not suppress enzyme synthesis, since not all tissues of the organism exhibit G-6-P.D. deficiency.

Formation of an abnormal enzyme was indicated by the following: *1*) Upon separation of erythrocytes (differential osmotic hemolysis) into young and old cells, G-6-P.D. deficient cells had a 30-fold decrease in enzyme in the older fraction, while normal erythrocytes had only a 10-fold decrease in enzyme. *2*) Upon incubation at 37° C., hemolysates of enzyme deficient cells lost all G-6-P.D. activity in two to four hours, while hemolysates of normal cells retained 70 to 90 per cent of their activity at four hours. *3*) Stability of G-6-P.D. in hemolysates of deficient cells could be markedly enhanced by addition of triphosphopyridine nucleotide. *4*) No inhibitor of G-6-P.D. was found in hemolysates of enzyme deficient cells.

These data strongly suggest that in erythrocyte G-6-P.D. deficiency the abnormal gene leads to the production of an altered enzyme differing in stability from normal G-6-P.D. As a consequence, in red cells, which may synthesize no protein, the activity of this enzyme might decrease more rapidly than normal with cell aging.

Inhibition of Virus Multiplication by Transferrin. CHRISTOPHER M. MARTIN and JAMES H. JANDL,* Boston, Mass.

Two observations prompted the hypothesis that transferrin possesses anti-infection properties: *1*) A. L. Schade demonstrated that transferrin suppresses the growth of many pathogenic and nonpathogenic bacteria; unlike the cells of higher animals, many bacteria cannot utilize transferrin-bound iron. *2*) Studies on four patients with agammaglobulinemia disclosed that all four had low serum iron levels and high serum iron-binding capacities (transferrin) refractory to iron administration; conceivably this chemical pattern represented a compensatory defense mechanism.

With highly purified human transferrin, the *in vitro* findings of Schade were confirmed; *in vivo*, however, only a weak antibacterial effect in rats and mice was demonstrated. More striking was an inhibition of common pathogenic viruses that occurred in tissue cultures containing transferrin. At pH 7.25 to 7.5, the cytopathogenic effect of 10 to 1,000 $TCID_{50}$ of ECHO, Coxsackie B, poliomyelitis Types 2 and 3, and adenoviruses was completely suppressed for five to seven days in the presence of transferrin at concentrations of iron-binding capacity equal to those in the plasma of iron-deficient patients. Multiplication of poliomyelitis Type 1, vaccinia and herpes simplex viruses was not affected. Transferrin principally inhibited viral synthesis; viral attachment to susceptible cells was inhibited to a minor degree. Transferrin did not bind to viruses, and was not virucidal. Iron salts reversed the antiviral effect only slightly; several cations of the transitional elements series were considerably more effective.

Thus far, eight metal-binding compounds have been screened for transferrin-like antiviral activity; of these, one—*d*-penicillamine—is moderately active *in vitro*.

The findings offer evidence that through metal-binding, transferrin: *1*) may function as an anti-infection mechanism *in vivo;* and *2*) inhibits a rate-controlling, metal-dependent reaction in the synthesis of viruses.

The Relationship between Genotype and $Rh_o(D)$ Antigen Sites on the Red Blood Cell. S. P. MASOUREDIS, Pittsburgh, Pa. (Introduced by Jonas E. Salk).

The antigens on the surface of the RBC represent immunochemically specific molecules whose synthesis is genetically determined. If the one gene-one product hypothesis applies to this model, an individual genetically homozygous would possess twice as many antigen sites on his RBC as would an individual who was genetically heterozygous. I-131 labeled incomplete anti-$Rh_o(D)$ provides a means of directly quantitating the number of antigen sites on $Rh_o(D)$ containing RBC.

High titered antisera have been fractionated by cold ethanol techniques, trace iodinated and the labeled isoantibody recovered by absorption and elution from $Rh_o(D)$ stroma. RBC are reacted at 37° C. for one hour with the I-131 anti-$Rh_o(D)$, washed repeatedly and then assayed for I-131. The quantity of isoantibody nitrogen bound to each RBC can be determined from the specific activity of the I-131 anti-$Rh_o(D)$ and the number of red cells.

The nitrogen bound to a random sample of RBC obtained from the donor population of the blood bank follows a bimodal distribution. One peak occurs at a "relative" nitrogen value of 1.10×10^{-10} μg. per cell and the other at 1.90. Of the 100 RBC studied, 83 per cent are associated with the 1.10 peak and the remaining 17 per cent with the 1.90 peak.

The RBC of 13 children from families with a history of Rh incompatibility, who are genotypically heterozygous, have a relative nitrogen value of 1.10 to 1.20. Their fathers' RBC yield a bimodal distribution similar to the one observed for the random population.

Although these preliminary results indicate that the two cell populations represent RBC which are homozygous and heterozygous with respect to the $Rh_o(D)$, gene, there is some scatter in the data with some nitrogen values between the two peaks. These findings suggest that the $Rh_o(D)$ antigen is heterogeneous and results in

the perturbation of the quantitative one-to-one relationship.

In Vitro Labeling of Granulocytes with DFP^{32}. ALVIN M. MAUER, JOHN W. ATHENS, HELEN ASHENBRUCKER and GEORGE E. CARTWRIGHT,* Salt Lake City, Utah.

The purpose of this paper is to describe a relatively simple method for studying the turnover of granulocytes out of blood. The labeling of granulocytes *in vitro* has been accomplished by drawing 500 ml. of whole blood into a plastic donor bag containing acid-citrate-dextrose (ACD) solution as an anticoagulant and incubating the blood with 150 μg. of DFP^{32} for one hour. At the end of this time the labeled blood is infused back into the subject. Blood samples are drawn subsequently, the leukocytes isolated and the leukocyte radioactivity determined.

Maximal labeling of the leukocytes in the bag is reached within 45 minutes. Plasma obtained from blood incubated for one hour with DFP^{32} did not cause *in vivo* labeling after infusion back into the subject. There appears to be no elution of the label or damage to the cells.

The leukocyte radioactivity in the blood after *in vitro* labeling decreased in an exponential fashion with a mean half-time of 6.5 hours in 10 normal subjects. The mean daily turnover of granulocytes out of blood in these 10 subjects was 77×10^9 cells. The exponential decrease in radioactivity suggests that granulocytes leave the blood as a result of random factors. Within the limits of the method, no evidence for return of granulocytes from extravascular pools was found.

The usefulness of this method can be illustrated in the following ways. Granulocytes stored for 24 hours at 4° C. were removed from the circulation within minutes. The granulocyte turnover rate in a patient with pneumococcal empyema was 210×10^9 cells per day; in a patient with chronic myelocytic leukemia, the turnover rate was 180×10^9. This method should be useful in studying the turnover of leukocytes in various disease states, the preservation of leukocytes and, by cross transfusion studies, leukocyte compatibilities.

Assessment of Adrenocorticotropic Activity with Bacterial Pyrogen in Hypopituitary States. JAMES C. MELBY, Minneapolis, Minn. (Introduced by Wesley W. Spink).

Endotoxins of gram-negative bacteria injected into man and animals stimulate adrenal secretory activity in the presence of an intact pituitary. Blood samples for cortisol estimation were obtained before and at 30, 60, 120 and 180 minutes following an intravenous injection of 0.25 μg. of a lipopolysaccharide derived from *Salmonella abortus equi* (pyrexal) in five healthy adults, seven patients with hypopituitarism and five patients receiving exogenous steroid therapy. Fever and systemic reactions were terminated at the end of each experiment by an intravenous injection of the succinate ester of cortisol. Plasma cortisol levels increased to more than twice the control values at two hours in healthy subjects. Four patients with hypopituitarism had no rise in plasma cortisol, whereas three others demonstrated a delayed and gradual increase. Patients receiving exogenous steroids also demonstrated a similar delayed response. The magnitude of response in patients receiving exogenous steroids was inversely related to the duration of steroid therapy.

The data from these studies show that the injection of bacterial pyrogen can provide additional information for the assay of hypophyseal corticotropin. This assay has been helpful in the diagnosis of hypopituitary states.

Hemodynamic Patterns in Portal Cirrhosis Determined by External Scintillation Detection. ISMAEL MENA, LESLIE R. BENNETT and SHERMAN M. MELLINKOFF, Los Angeles, Cal. (Introduced by Joseph F. Ross).

A technique for estimating the cardioportal circulation time has facilitated the study of hemodynamic patterns in portal cirrhosis.

Scintillation detectors are placed over the heart and over the liver. Simultaneous recording is begun after an intravenous injection of radioiodinated serum albumin (RISA). Radioactivity over the liver reaches a maximum 25 seconds after the maximum radioactivity is reached over the heart. The time between the maximum radioactivity over the heart and the maximum radioactivity over the liver is defined as the cardioportal circulation time.

In 36 normal individuals the cardioportal circulation time was 25 seconds (S.D., three seconds).

In 63 patients with cirrhosis of the liver but without intractable ascites the mean cardioportal circulation time was 45.8 seconds (S.D., seven seconds).

In nine patients with side-to-side or end-to-end portacaval shunts the mean cardioportal circulation time was 17.7 seconds (S.D., 2.6 seconds), reflecting arterial blood flow only.

Thirteen patients with intractable ascites had a cardioportal circulation time of 17.7 seconds (S.D., 3.5 seconds); the shape of these curves was like those seen in patients with surgical portacaval anastamosis.

In eight other patients whose ascites responded to therapy very slowly the circulation times were of intermediate lengths, with an average of 29.5 seconds.

Three patients whose ascites was initially intractable were again tested several months later when they began to respond to therapy. At this point the cardioportal circulation times were in the 40 second range.

It is suggested that in uncomplicated cirrhotics and in those patients with easily manageable ascites a large splanchnic blood volume prolongs the circulation time.

With intractable ascites the hemodynamic pattern suggests that most of the blood reaching the liver is arterial.

Relation of Intracellular Ions to Certain Carbohydrate Metabolites and Energy Sequences in Kwashiorker. JACK METCOFF,* SILVESTRE FRENK and IRENA ANTONOWICZ, Chicago, Ill. and Mexico, D. F.

Kwashiorkor (severe protein and calorie malnutrition) is largely responsible for high child mortality rates in technically underdeveloped areas of the world. Previous chemical analyses of muscle from dying children revealed extracellular hyposmolarity and markedly increased intracellular H_2O and sodium content, $(Na)_{icw}$. $(K)_{icw}$ was preserved but concentration, $[K]_{icw}$, fell. Decreased effectiveness of some $[K]_{icw}$-dependent energy-yielding metabolic sequence required for active Na extrusion from the cell was inferred.

In this study, muscle biopsies were obtained twice from eight children with severe kwashiorkor, of whom three subsequently died. Extracts were analyzed for electrolytes, including phosphate fractions [total (P_T), organic (P_o), "apparent"—phosphocreatine P + inorganic—(P'_1), and "true" inorganic (P_1)] and Mg, as well as noncollagen nitrogen (NCN), pyruvate, lactate, alpha-ketoglutarate and citrate. Interrelationships of these organic metabolites and ions were expressed as ratios.

(P_T), largely $(P_o)_{icw}$, was reduced significantly in all children. $(Mg)_{icw}$ was slightly depleted. In contrast to children who recovered, muscle cells from children who died revealed decreasing $\frac{P_o}{\text{pyruvate}}$, but increasing

$$\frac{\text{lactate}}{\text{pyruvate}}, \quad \frac{\text{citrate}}{\text{pyruvate}}, \quad \frac{\text{citrate}}{\alpha\text{ketoglutarate}}, \quad \frac{\text{Mg}}{\alpha\text{ketoglutarate}} \text{ and}$$

$$\frac{P'_1}{\alpha\text{ketoglutarate}}.$$

$(Citrate)_{icw}$ did not accumulate.

There variations imply either diminished Krebs' Cylce activity or augmented lipogenesis, the latter concordant with the fatty livers and conservation of subcutaneous fat found in severe kwashiorkor.

Increased intracellular (P'_1), and $(Na)_{icw}$, relative to pyruvate, simultaneously with decreased $[K]_{icw}$, suggest reduced utilization of high-energy phosphate derived from phosphoenol pyruvate. Since Na is a specific inhibitor (*in vitro*) of that reaction, some accumulation of cofactors $(Mg)_{icw}$ and $(K)_{icw}$ would be expected and was noted.

The significance of altered concentrations of intracellular electrolytes was clarified when related to simultaneous change of glycolytic intermediates in muscle. This study supports the hypothesis that death in kwashiorkor results from characteristic defects in electrolyte dependent, energy-yielding metabolic sequences.

Identification of the Vitamin B_{12} Binding Protein of Normal and Chronic Myelogenous Leukemia Serum. AARON MILLER and JOHN F. SULLIVAN, Boston, Mass. (Introduced by James M. Faulkner).

The seromucoid fraction (SF) of serum, including acidic mucoproteins such as orosomucoid, has been shown to bind large amounts of added cyanocobalamin (B_{12}) in chronic myelogenous leukemia (CML). In the present study the possibility that this fraction contains the normal B_{12} binding protein ($B_{12}BP$) was investigated.

Serum was electrophoresed (pH 4.5, acetate buffer) with a starch block. Protein, sialic acid and B_{12} concentrations were determined on eluates from 1.0 cm. segments of the block. *Euglena gracilis* assay was used for B_{12} measurements. SF was prepared by Winzler's method.

The SF contained an average of 78 per cent (range, 67 to 100 per cent) of B_{12} of six normal sera. In three CML sera, average B_{12} concentration was 10.11 mμg. per ml. (range, 6.10 to 15.00 mμg. per ml.), with 98 to 100 per cent found in SF. On electrophoresis of serum at pH 4.5, albumin and globulins remained at the origin or migrated cathodally. A small protein and sialic acid peak (orosomucoid) was found 2 to 4 cm. anodal to the origin in normal sera, where most B_{12} was also found (mean, 84 per cent; range, 67 to 94 per cent). B_{12} was found in Cohn Fractions I through VI, Fraction V having the highest concentration (29 per cent) of original plasma B_{12}. Paradoxically, Fraction VI, containing acidic mucoproteins including orosomucoid, had little of the original plasma B_{12} (11 per cent). Mobility of $B_{12}BP$ of CML serum was similar to normal (97 to 98 per cent of the B_{12} found anodally). Added Co^{60} B_{12} bound *in vitro* to CML serum was found at the same site as endogenous vitamin.

Hence, $B_{12}BP$ of normal serum has been identified as a constituent of serum SF and as an acidic protein with electrophoretic mobility similar to orosomucoid. However, it was not selectively precipitated with the other acidic proteins of Cohn's Fraction VI. $B_{12}BP$ of CML serum has properties similar to normal $B_{12}BP$, suggesting that the great increase in $B_{12}BP$ in CML serums results from increased normal $B_{12}BP$ rather than to an abnormal protein.

Effect of Insulin and Anaerobiosis on the Membrane Transport and Intracellular Metabolism of Glucose in Diabetic Muscle. H. E. MORGAN, D. M. REGEN and C. R. PARK,* Nashville, Tenn.

When the heart from a normal rat was perfused with bicarbonate buffer containing 100 mg. per cent glucose, the glucose uptake was 4.3 mg. per Gm. per hour and glucose space was 297 μl. per Gm. Addition of insulin to the normal heart *in vitro* increased glucose uptake to 10.0 mg. per Gm. per hour and the glucose space to 458 μl. per Gm. without any change in the sorbitol or extracellular space (370 μl. per Gm). These findings indicate that glucose uptake is normally limited by transport of sugar through the cell membrane and that insulin stimulates transport to the extent that intracellular phosphorylation becomes rate limiting as indicated by the accumulation of free intracellular glucose. The glucose uptake of the heart from an alloxan diabetic animal was depressed to 1.6 mg. per Gm. per hour and free intracellular sugar did not accumulate as evidenced by a glucose space of 360 μl. per Gm. Membrane transport, therefore, appears to be the first point at which glucose uptake is inhibited by this condition. The addition of insulin *in vitro* increased the glucose uptake only to

3.1 mg. per Gm. per hour, but a large accumulation of free intracellular sugar was observed (glucose space, 621 μl. per Gm.). These data show the presence of a second metabolic block at the phosphorylation level. The anaerobic perfusion of the diabetic heart increased the glucose uptake to 4.9 mg. per Gm. per hour, and no free intracellular glucose was found, indicating that both transport and phosphorylation of glucose had been stimulated. When insulin was added under anaerobic conditions the glucose uptake increased greatly to 12.2 mg. per Gm. per hour and still no intracellular hexose was present (glucose space, 365 μl. per Gm.). These data indicate that insulin increases only the membrane transport of glucose in the diabetic muscle whereas anaerobiosis increases both the transport and phosphorylation capacity. The anaerobic effect offers an explanation for the effect of exercise on the glucose uptake of diabetic muscle.

The Volume of Distribution of Lactose in the Body Fluids and the Renal Clearance of Lactose. ASHTON B. MORRISON, Philadelphia, Pa. (Introduced by Truman G. Schnabel, Jr.).

The volume of distribution of such carbohydrates as inulin and sucrose in the body fluids of man and animals has been extensively studied and the manner in which such substances are excreted by the kidneys is well understood. Little is known concerning the distribution of lactose; this is all the more surprising as it is a carbohydrate which is synthesized, at any rate in the female, under physiological circumstances. This omission has been partly the result of the lack of a satisfactory method for the estimation of lactose. The introduction of a specific method for the analysis of lactose depending on its reaction with methylamine in the presence of alkali has permitted a study of the lactose space in the dog.

Known amounts of lactose were injected intravenously into previously nephrectomized dogs. After periods of time lasting up to 24 hours, samples of serum were withdrawn and analyzed for lactose. The volume of distribution of the lactose was calculated in each animal and ranged from 17.2 to 18.0 per cent of the body weight three hours after the injection and from 18.2 to 21.3 per cent of the body weight six hours after the injection. No lactose entered the cerebrospinal fluid in measurable quantities. In three anesthetized dogs, the renal clearances of lactose and exogenous creatinine were measured and found not to differ significantly from one another.

Quantitative Analyses of Enzymes in Glomeruli and Renal Tubular Cells. ROBERT C. MUEHRCKE, VICTOR E. POLLAK and SJOERD L. BONTING, Chicago, Ill. (Introduced by Robert M. Kark).

Our purpose is to investigate the role played by enzymes in urine formation by nephrons of normal and diseased kidneys, with particular emphasis on *1*) tubular glucose reabsorption, and *2*) potassium deficiency.

Renal biopsy tissue was frozen rapidly in liquid nitrogen and sectioned. The anatomic units of the nephron were identified accurately in stained sections. The adjacent unstained lyophilized section was dissected out, using microscalpels. The minute fragments of glomeruli, of proximal, of distal and of collecting tubules, were weighed accurately on a quartz-fiber balance (sensitivity, 4×10^{-10} Gm.). Sample weights ranged from 10^{-8} to 10^{-7} Gm., corresponding to 20 to 200 cells. The tissues were transferred to microtest tubes for incubation, enzyme activities being determined quantitatively by ultramicro-biochemical techniques.

Alkaline phosphatase (AP) and lactic dehydrogenase (LDH) activities were determined quantitatively in kidney specimens from six healthy adults and from patients with hypophosphatasia, cystinosis, adult Fanconi syndrome, familial renal glycosuria and hypokalemia (associated with renal or gastrointestinal potassium loss). AP and LDH were also studied in canine kidneys before and after phlorizin-induced glycosuria, and in rat kidneys before and during experimental potassium depletion.

The data indicate that: *1*) Enzyme activity can be measured quantitatively in individual functional units of the nephron. *2*) In some diseases no correlation was found between tubular morphologic changes and changes in enzyme activity. *3*) A parallel and uniform decrease of both enzymes occurred only in Fanconi syndrome, suggesting a nonspecific effect on tubular enzymes. *4*) AP activity was virtually absent in proximal tubules of some patients with *no* glycosuria, suggesting AP is not a key enzyme for glucose reabsorption. Moreover, AP activity in canine proximal tubules was not affected by phlorizin. *5*) The specific morphologic lesions of hypokalemia were seen in collecting tubules. LDH activity was markedly decreased only in the papilla from hypokalemic patients and potassium deficient rats.

Renal Autoexplantation and a Functional Characterization of Nonexcretory Renal Tissue. E. E. MUIRHEAD,* J. A. STIRMAN and FRANCES JONES, Dallas, Texas.

Bilateral nephrectomy is associated with hypertension, cardiovascular disease and hemolysis. Autoexplantation of renal tissue has been used in the dog to evaluate protection against these abnormalities by nonexcretory renal tissue.

Technique I (eight dogs). Renal medulla from vascular arcade to papillary tip was finely divided in a blendor, washed twice with Gey's solution and injected intravenously through an 18 gauge needle in a suspension containing antibiotics. The procedure required 15 minutes. *Technique II.* The whole kidney in one group (eight dogs) and the outer medulla in another (eight dogs) were similarly treated and injected intraperitoneally.

Two to seven weeks later the contralateral kidney was removed; the animal received protein by diet (about 3 Gm. per Kg. per day) and saline by vein (16 ml. per Kg. per day) for four days. Control nephrectomized dogs received the same dietary and sodium intake. Arterial pressure and erythrocytic life span were measured. Vis-

ceral arterioles were appraised for necrosis and alterations in wall to lumen ratio.

Tubular epithelium was found within pulmonary vessels, pulmonary parenchyma and within nodules studding the peritoneum. Granular and clear cells formed tubules and clusters, clear cells predominating. Juxtaglomerular cells were not identified.

Erythrocytic life span was markedly shortened following renal ablation (nephrectomy *vs.* normal, $p < 0.01$) but not in the presence of renal explants (explant *vs.* nephrectomy, $p < 0.001$). Accelerated hypertension did not occur in the presence of renal explants (Δ arterial pressure: explant *vs.* zero, p 0.4; nephrectomy *vs.* zero, p 0.001; explant *vs.* nephrectomy, p 0.001). Myocardial and arteriolar necrosis were less common in explanted than in nephrectomized animals. Arteriolar wall to lumen ratio was prominently elevated in the nephrectomized group (p *vs.* normal, < 0.001) while in explant groups the ratio appeared normal (p 0.06).

The experiments support the concept that nonexcretory renal tissue protects against hypertensive cardiovascular disease and erythrocytic injury. Renal medulla appears to contribute to these functions.

Corticosteroid Synthesis and Release by Human Adrenal Slices. PATRICK J. MULROW and GEORGE L. COHN, West Haven and New Haven, Conn. (Introduced by Philip K. Bondy).

In vivo studies of human adrenocortical hormones are influenced by conditions affecting their secretion, metabolism and excretion. Adrenalectomy offers a direct experimental approach to the study of steroid synthesis *in vitro*. Four "normal" glands were surgically removed from three patients with neoplastic disease. The glands were immediately iced, freed of fat, sliced and incubated in Krebs-Ringer bicarbonate buffer with added glucose in an atmosphere of 95 per cent O_2, 5 per cent CO_2. Progesterone-4-C^{14} or corticosterone-4-C^{14} was added to some experimental flasks. A dried, alkaline washed chloroform extract of the medium was applied to a paper chromatogram in the toluene-propylene glycol system. The eluted cortisol and cortisone areas were then chromatographed in the Bush-C system. Cortisol was always found. Cortisone and aldosterone were occasionally detected. Progesterone-4-C^{14} was incorporated into cortisol (specific activity, 0.18 to 0.66 μc. per mg.) but radioactive aldosterone could not be detected. Corticosterone-4-C^{14} was incorporated into aldosterone (S.A., 1.0 to 2.1 μc. per mg.) and cortisol (S.A., 0.058 μc. per mg.). This observation suggests that a minor pathway exists for the synthesis of cortisol from corticosterone.

A 12.5 Gm. hyperplastic gland and a 27 Gm. adenoma were removed from two patients with Cushing's syndrome. Tissue slices were studied in a similar manner. The yield of cortisol per gram of tissue was equivalent to that released by "normal" glands. This finding suggests that the excessive secretion of cortisol in these two subjects resulted from the large size of the gland and not from overactivity of the cells. The adenoma incorporated progesterone-16 H^3 into cortisol. Only a minimal quantity of radioactivity was present in the aldosterone area.

These studies indicate that the human adrenal gland continues to synthesize and release corticosteroids *in vitro*. This method affords an opportunity to investigate directly human steroid synthesis, particularly in diseased adrenal glands.

Magnesium Excretion Studied by Stop-Flow Analysis. H. V. MURDAUGH, JR. and R. R. ROBINSON, Birmingham, Ala. and Lackland A.F.B., Texas. (Introduced by William M. Nicholson).

Magnesium excretion in anesthetized dogs was studied using stop-flow analysis as described by Malvin, Sullivan and Wilde during osmotic diuresis using eight minute occlusion. Attempts using chemical analysis for magnesium were not successful due to sample size. Accordingly, Mg^{28} was used in six dogs administered with the inulin and PAH three minutes before release of occlusion or by constant infusion attaining constant blood level prior to and during occlusion. When given as a single injection Mg^{28} appeared in distal nephron samples showing that magnesium could reach distal nephron lumen without filtration, but the appearance curve was compatible with diffusion. When given by constant infusion magnesium U/P and $\frac{\text{Magnesium U/P}}{\text{Exogenous creatinine U/P}}$ were determined. Active reabsorption of magnesium by proximal portion of distal nephron was demonstrated by magnesium U/P as low as 0.33 in these samples. $\frac{\text{Magnesium U/P}}{\text{Exogenous creatinine U/P}}$ decreased in proximal portion of distal nephron, increased in the more distal nephron, but did not exceed 1.0 even with magnesium loading. The difference in

$$\frac{\text{Magnesium U/P}}{\text{Exogenous creatinine U/P}}$$

in proximal and distal portions of distal nephron was as great as 0.08 and 0.45, respectively. Correction for determined protein binding increased this difference. These data suggest magnesium addition to the distal nephron after active reabsorption has occurred proximally, but do not completely exclude diffusion.

A New Clinical Entity in Patients Adrenalectomized for Cushing's Syndome. DON H. NELSON * and J. WILLIAM MEAKIN, Boston, Mass.

Since describing a patient with Cushing's syndrome who, following bilateral adrenalectomy, developed an adrenocorticotropic hormone (ACTH) secreting tumor of the pituitary gland, we have studied two similar patients. Plasma samples, for ACTH determination, from 11 additional patients who may have a similar condition, have been supplied by other investigators and are being studied. In none of our three patients was there evidence of a pituitary tumor by X-ray examination prior to adrenalectomy. Objective evidence of a pituitary tumor appeared two to eight years following adrenalectomy for hyperplasia.

These patients were much more deeply pigmented than the average adrenalectomized or Addisonian patient, and had elevated plasma ACTH levels which were not completely suppressed with 20 mg. of cortisol administered intravenously over a four hour period which always produced suppression in a group of Addisonian patients. Histology of the pituitary in the first patient resembled most closely a chromophobe adenoma. The mean level of ACTH activity in the plasma of unstressed Addisonian patients was found to be 8 mU per 100 ml. plasma with a range of 0 to 30 mU. ACTH levels in the plasma of the three patients studied have reached 400, 74 and 61 mU per 100 ml., respectively. There were no determinations of ACTH prior to adrenalectomy in these patients. Attempts to measure ACTH preoperatively in the plasma of other patients with Cushing's syndrome have been unsuccessful. Failure to find ACTH in the plasma of untreated patients with Cushing's syndrome does not prove that ACTH is not present in increased amounts, however, but only that it is not elevated sufficiently to be measured by the method used.

Active Sodium Transport by the Proximal Tubule of Necturus Kidney. DONALD E. OKEN, GUILLERMO WHITTEMBURY, ERICH E. WINDHAGER, HANS J. SCHATZMANN and ARTHUR K. SOLOMON, Boston, Mass. (Introduced by James P. O'Hare).

Bidirectional sodium fluxes across the single proximal tubule of Necturus kidney were measured *in situ*. In 10 stopped flow microperfusion experiments, perfusion fluid, essentially isosmotic with Necturus plasma, contained 100 mEq. per L. NaCl, and tracer amounts of Na^{24} and C^{14} inulin. Plasma Na concentrations, and C^{14} inulin, Na^{24} and total sodium concentrations of injected and collected solutions were determined. Mean water efflux for the 20 minute periods of perfusion was 27 per cent, the reabsorbate containing essentially the same Na concentration as Necturus plasma. Net sodium efflux occurred at a rate of 62 $\mu\mu$Eq. per cm.^{2} per second. Na influx and total Na efflux were 204 $\mu\mu$Eq. per cm.^{2} per second and 266 $\mu\mu$Eq. per cm.^{2} per second. Measurement of transtubular electrical potential difference gave a mean value of 20 mV. (lumen negative). This net sodium transport against a combined electrical and chemical potential gradient is evidence of active transport. The active Na flux is apparently discharged through a "leaky sieve," since some 75 per cent of the Na transported outwards seems free to return into the tubule.

Studies of the Influences of Steroid Hormones on the Renal Excretion of Sodium. MAURICE M. PECHET* and EVELYN L. CARROLL, Boston, Mass.

The effects of steroids on renal excretion of sodium were investigated with metabolic balance studies in panhypopituitary and Addisonian subjects. Sodium rentention induced by the administration of 11-desoxycorticosterone is diminished by 1,2-dehydrogenation of the parent steroid. Administration of 3 mg. of 11-desoxycorticosterone daily to an Addisonian subject causes an average rate of sodium retention of 43.94 ± 5.22 mEq. per 24 hours. The same dose of 1,2-dehyrdo-11-desoxycorticosterone causes an average rate of sodium retention of 25.76 ± 3.90 mEq. per 24 hours. Changes in glomerular filtration rate were minimal with both steriods. Whereas 17α-hydroxylation of 11-desoxycorticosterone results in a substantial loss of sodium-retaining properties of the parent steroid, 16α-hydroxylation results in a very marked loss.

Sodium retention induced by the administration of 11-desoxycorticosterone is reduced slightly by concomitant administration of 16α-hydroxy-11-desoxycorticosterone (dose ratio, 1 to 14). Sodium retention associated with the administration of 9α-fluoroprednisolone is not decreased by concomitant administration of 16α-methyl-17α-hydroxy-11-desoxycorticosterone (dose ratio, 250 to 1).

In Addisonian subjects: *1*) the administration of 30 mg. of 9α-fluoro-16α-hydroxyprednisolone daily induces an average rate of sodium excretion of 16.27 ± 2.52 mEq. per 24 hours; with the addition of 150 μg. of 9α-fluoroprednisolone daily, the average rate of excretion drops to 13.17 ± 3.17 mEq. per 24 hours; *2*) the administration of 30 mg. of 16β-methylprednisone daily induces an average rate of sodium excretion of 3.49 ± 0.255 mEq. per 24 hours; with the addition of 150 μg. of 9α-fluoroprednisolone daily, the rate of excretion is 5.88 ± 0.11 mEq. per 24 hours.

The sodium-retaining properties of the naturally occurring steroid 11-desoxycorticosterone are substantially diminished by 1,2-dehydrogenation, 11β-hydroxylation, and 17α-hydroxylation, whereas 16α-methylation, 16β-methylation, or 16α-hydroxylation negate the sodium-retaining properties; this cannot be ascribed to complete inactivation of the steroid since 16α-methylation, 16β-methylation and 16α-hydroxylation negate the sodium-retaining properties of 9α-fluoroprednisolone, leaving intact the antianabolic and anti-inflammatory properties.

Studies of Human Myoglobin. G. T. PERKOFF, H. C. SCHWARTZ, R. L. HILL and F. H. TYLER,* Salt Lake City, Utah.

The demonstration that structural abnormalities of hemoglobin occur in the hereditary hemoglobinopathies suggested to us that similar abnormalities might be present in myoglobin in the genetically determined muscular dystrophies. We have compared several characteristics of normal myoglobin with myoglobin from muscular dystrophy patients. Myoglobin was prepared by differential solubility in 3 M phosphate buffer, a procedure which completely separates contaminating hemoglobin.

On paper electrophoresis, the myoglobins had approximately one-third the mobility of hemoglobin. Free electrophoresis (veronal, pH 8.6) yielded a major myoglobin peak at 2.4 to 2.8×10^{-5} μ, with less constant minor peaks. In this buffer, hemoglobin has an electrophoretic mobility of 3.0×10^{-5} μ.

The sedimentation constant of normal myoglobin was

1.91 S_{20}, W_o. The constant for hemoglobin is 4.17 to 4.24 S_{20}, W_o.

Normal myoglobin and myoglobin from a patient with facioscapulohumeral dystrophy digested completely with trypsin, pH 8.0, 37° C. Under these conditions, hemoglobin leaves a large insoluble core. Fingerprints (Ingram) of these myoglobins were similar and showed 24 to 28 peptides. The pattern is grossly different from that of hemoglobin, although the number of peptides is similar.

Studies of heme synthesis *in vitro* with radioiron showed that both normal and dystrophic muscle extracts could incorporate small amounts of iron into myoglobin. The characteristics of this process appear to be similar to those by which heme is synthesized in nucleated red blood cells and other tissues. Although no distinct differences among the various myoglobins have been demonstrated thus far these studies are being continued.

Steroid Effects on Galactose Metabolism. LeRoy A. Pesch, Stanton Segal and Yale J. Topper, Bethesda, Md. (Introduced by J. E. Rall).

Progesterone, androsterone and testosterone have been shown to accelerate the oxidation of D-galactose-1-C^{14} to $C^{14}O_2$ when added *in vitro* to surviving rabbit liver slices or small intestinal segments. A soluble fraction derived from rabbit liver homogenate responds to the steroids in the same manner as the liver slice.

In an attempt to evaluate the physiological significance of these findings, the effect of progesterone treatment on the incidence of galactose induced cataracts in rats has been investigated. Daily 2.5 mg. injections of progesterone caused a three day delay in the time for 50 per cent development of mature cataracts in rats receiving a 40 per cent galactose diet and a 12 to 14 day delay in the time for 50 per cent development of mature cataracts in rats receiving a 30 per cent galactose diet. No effect of progesterone on cataract development was observed in rats receiving a 30 per cent xylose diet.

The effect of progesterone has also been studied in one prepubertal patient with congenital galactosemia. Two μc. (0.42 mg.) of D-galactose-1-C^{14} was administered intravenously before and after a six day treatment period with daily 10 mg. intramuscular injections of progesterone. Samples of expired air were obtained during the six hour period immediately following administration of the isotope, and $C^{14}O_2$ content was determined. No detectable amount of $C^{14}O_2$ appeared in the expired air prior to progesterone treatment. However, following progesterone treatment a significant fraction of the administered isotope appeared as $C^{14}O_2$ in the expired air. Further studies are being conducted to extend these preliminary observations and to evaluate possible therapeutic implications in congenital galactosemia.

The Physical Properties of Arteries and Their Variation In Vivo. Lysle H. Peterson * and Roderick E. Jensen, Philadelphia. Pa.

A precise, quantitative and mechanistic knowledge of the behavior of the physical properties of blood vessels is essential for an understanding of the behavior of the cardiovascular system. The processes determining vascular regulation and resistance in health and in diseases such as hypertension are direct functions of these properties, *viz.*, elastic, viscous and inertial characteristics. Methods have been devised and applied which record (on magnetic tape) instantaneous, intravascular pressure (to ± 0.5 mm. Hg) and vessel wall circumference (to ± 0.001 mm.) from multiple sites in the vasculature of intact, living dogs under a variety of circumstances. From these data stress-strain relationships are analyzed by digital and analogue computer techniques. Equations describing the physical properties of the blood vessels are thus derived and precise values for coefficients of vessel elasticity, viscosity and mass are determined.

Stimulation of the autonomic nerve supply, or application of the respective neurohumors, to the arterial system results in variations of the elasticity and viscosity of blood vessels by as much as 400 per cent. Sympathetic nerve activity or norepinephrine causes the values to increase while acetylcholine causes the values to decrease. Epinephrine causes various changes in various sites. The normal loading effect of the tissue surrounding the vessels (except contracting muscle) is small. Although the magnitudes of these variations of mechanical properties are large and do profoundly affect the behavior of the cardiovascular system the magnitude of the absolute changes in arterial wall motion are small (< 5 per cent) compared to the "unstressed" circumference of the blood vessel. This finding, *i.e.*, that "small strain" theory can be applied to blood vessels, is of major importance in the analytical approach to understanding the mechanical properties of the vascular system.

These results imply that the concept of pressure regulation via intravascular neuroreceptors is probably misleading since the displacement (stretch) of these receptors is determined by the elasticity and viscosity of the vessel wall as well as by intravascular pressure.

Hematological Studies of X-Irradiated Rabbits. Sergio Piomelli and Marcus S. Brooke, Boston, Mass. (Introduced by Kendall Emerson, Jr.).

Rabbit red blood cells labeled *in vitro* with Cr^{51} were injected into the donors: the apparent half-life was 18 ± two days. The fate of the erythrocytes was studied following irradiation either at 600 r. ($LD_{40/14}$) or at 1,100 r. ($LD_{100/14}$) delivered as a split dose of 600 r. followed by 500 r. 24 hours later. Red blood cell mass determinations were done at weekly intervals using K^{42}. Twenty-four hours after irradiation there was an 8 to 10 per cent decrease in the chromium count per ml. of blood, with a simultaneous increase in the total erythrocyte volume of approximately 8 per cent in the animals exposed to 600 r. and 15 per cent in those exposed to 1,100 r. The Cr^{51} counts per ml. of blood then remained unchanged for six to eight days in the 600 r. group and four to six days in the 1,100 group. During this period

the total erythrocyte volume in the 600 r. group remained unchanged. In both the 600 r. and the 1,100 r. group the rate of disappearance of Cr-labeled cells then increased over that found prior to irradiation. This accelerated disappearance continued for approximately 10 days in the former group and until death in the latter and was accompanied by diffuse hemorrhages and the appearance of Cr^{51} in the feces. The total erythrocyte mass declined during this period. These results suggest that immediately following mid-lethal or lethal X-irradiation there is an outpouring into the circulation of unlabeled erythrocytes, with, in the case of lethally irradiated animals, a concomitant release of old sequestered Cr^{51}-labeled cells. In both instances there is then a period when there is neither production nor destruction of erythrocytes. These findings will be discussed in relation to the functional capacity of the reticulo-endothelial system in X-irradiated animals.

Application of Automatic Processing Techniques to Medical Data. I. The Electrocardiogram. HUBERT V. PIPBERGER, LEONARD TABACK, ETHEL MARDEN, HENRY L. MASON and EDWARD D. FREIS,* Washington, D. C.

A facility for automatic processing and analysis of medical data has been assembled. The electrocardiogram was chosen as the initial pilot example to determine the feasibility of this approach. Machine analysis of such data is completely objective. Large numbers of records can be processed with high speed and analytic procedures too tedious for routine use can be incorporated. Thus, large-scale statistical studies or mass screening projects are facilitated.

ECG leads (Schmitt's orthogonal SVEC III) are recorded simultaneously through FM channels on magnetic tape. Records can be displayed repeatedly in scalar or vectorial form by use of the play-back mechanism. These can then be studied in detail for development and testing of new analytic techniques suitable for computer processing.

Equipment has been designed to convert the ECG information as recorded on magnetic tape into numerical form for further processing through digital electronic computers. The converted records then are also stored on magnetic tape. The data are converted from analogue to digital form at a sampling rate of 1,000 per second. This conversion process has been tested successfully by retrieval of the tracings in their original form after passage through the computer. The computer program reduces the ECG information into a single time-varying vector in three-dimensional space for analysis of both magnitude and orientation. This type of data combines the information of scalar electrocardiograms and spatial vectorcardiograms.

The described method of ECG data processing can be applied with some modifications to other medical data such as phonocardiograms, pressure and flow curves, electroencephalograms and others. A diagnostic computer program can also be developed by adding historical and physical data obtained from patients.

The Use of Bromelin in the Detection of Erythrocyte Antibodies. BERNARD PIROFSKY, Portland, Ore. (Introduced by Edwin E. Osgood).

Studies were made of a group of enzymes derived from the pineapple and available in a concentrated form as bromelin. Preliminary investigation indicated that isotonic solutions of this material at a concentration of 0.5 per cent buffered to pH 5.5 would induce rapid agglutination when directly mixed with sera containing erythrocyte antibodies and erythrocytes. The prepared reagent was stable at 4° C. and was used to devise an immediate and a 15 minute bromelin test. Comparison of these procedures with an indirect Coombs' test and a papain-cysteine test revealed that the bromelin tests in general were more sensitive, with sharp end-points. The bromelin tests were found to have a wide range of activity and were used to detect antibodies directed against the ABO, Rh-hr, MNS, P, Lewis, Kell, Kidd, Duffy, Vel and Sutter blood groups. Because the immediate bromelin test eliminated washings, incubation and pretreatment of erythrocytes, a tube cross-match giving rapid results was devised. This test was found to have a universal action, demonstrating incomplete, saline, warm-acting, cold-acting, natural and immune antibodies. The practicality of such a rapid standard, one-tube cross match will be discussed.

The bromelin reagent was utilized to detect auto-antibodies coating erythrocytes in 11 cases of acquired hemolytic anemia. An immediate and a 15 minute bromelin test were devised and found to be effective in all cases. Studies will be presented demonstrating that the bromelin reaction in relationship to auto-antibodies is dependent on a labile plasma factor.

Bone Marrow Hemolysis in Anemia. MYRON POLLYCOVE, Berkeley, Cal. (Introduced by Paul M. Aggeler).

Little is known concerning anemia without reticulocytosis, yet with marrow erythroid hyperplasia, in patients unresponsive to all known therapy except transfusions of blood. The apparent discrepancy between erythroid hyperplasia on the one hand and blood findings of aregenerative anemia on the other, may be explained by: *1*) nonuniform marrow involvement, *2*) prolongation of erythron maturation time, and *3*) hemolysis in marrow of maturing erythrons. This study was undertaken to characterize and quantitate the functional defect in this puzzling anemia.

Sixteen such patients and 12 normal subjects were studied by intravenous injection of plasma-bound radioiron and examination of blood and marrow cytology. P^{32} red cell volume and net incorporation of radioiron into red cells were determined. Daily hemoglobin synthesis corresponding to erythron fixation of iron was determined by plasma radioiron analysis. Marrow uptake and release of radioiron, splenic sequestration and destruction of erythrocytes, and storage deposition of radioiron in liver were measured with surface scintillation counters.

Myeloid-erythroid ratio in marrow was greatly decreased to a mean of 0.7 (normal, 2.7). Though incorporation of radioiron into marrow erythrons was rapid, in striking contrast to typical hemolytic anemias, marrow release was markedly delayed, there was associated re-entry of radioiron into plasma with hepatic deposition, and very little radioiron in circulating erythrocytes. Daily hemoglobin synthesis was increased approximately fourfold. Erythron life span was greatly shortened to a mean of 18 days (normal, 117), including very short-lived erythrons destroyed in marrow and relatively long-lived circulating erythrocytes. Splenic sequestration and destruction of erythrocytes was relatively slight.

This study has demonstrated that erythropoiesis is markedly increased in these patients with erythroid hyperplasia, but largely ineffective due to erythron destruction in marrow. This functional defect was also observed in similarly studied patients with pernicious anemia, thalassemia major and acute erythremic myelosis, suggesting a lack or inactivation of enzymes or other factors essential for erythron maturation and survival.

Uptake and Release of Fatty Acids by Adipose Tissue. M. S. RABEN * and C. H. HOLLENBERG, Boston, Mass.

Liberation of unesterified fatty acids *in vitro* from rat epididymal, retrorenal and subcutaneous adipose tissue was stimulated by addition of epinephrine, porcine and human corticotropin, bovine and human growth hormone and by 0.5 mg. per ml. beta-mercaptoethylamine (cysteamine). Regular but smaller responses were obtained in human subcutaneous fat with epinephrine and with mercaptoethylamine but the effect of the pituitary preparations was inconstant and perhaps negligible under same conditions (Krebs-Ringer phosphate, 5 per cent albumin, 37° C., three hours in air). Fasting the rat sensitized the fat to growth hormone but 10 to 30 μg. per ml. was still needed in contrast to less than 0.1 μg. per ml. of corticotropin. *In vitro* and *in vivo* effects correlated well for epinephrine, but poorly for mercaptoethylamine and corticotropin which had no clear effect on plasma fatty acids and for growth hormone which raised plasma values with proportionately much smaller doses. The action of mercaptoethylamine was not simulated by mercaptoethanol, ethanolamine, thioglycollic acid or cysteine and may represent competition with coenzyme A.

Observations on the uptake of added oleic, palmitic and lauric acid by rat epididymal fat showed that little entered the fat from the albumin-containing medium at physiological concentrations of acid, in agreement with the unidirectional flow *in vivo*, but that at high concentrations (4 to 9 mEq. per L.) free fatty acids accumulated in the adipose tissue. Addition of glucose and insulin increased the loss of acids from the medium, but free acids were not found in the fat. Glucose and insulin thus appear to favor esterification in adipose tissue and may in this way suppress fatty acid mobilization since the acid product of lipolysis would be re-esterified. Glucose and insulin also reduced the corticotropin-induced release of fatty acids, and the effect on fatty acid disappearance was in turn inhibited by mercaptoethylamine.

Studies on the Blood-Cerebrospinal Fluid Barrier in Man. DAVID P. RALL, EDWARD MOORE, NATHAN TAYLOR and CHARLES G. ZUBROD,* Bethesda, Md.

Although the transfer of drugs from blood to cerebrospinal fluid (CSF) is important in experimental therapeutics, there has been no systematic study of the blood-CSF barrier in man. We have recently described the general principles of a standard method for studying the blood-CSF barrier in the dog. Constant plasma concentrations of various test drugs are maintained. Small amounts of CSF were removed serially from the cisterna magna and analyzed. Both the steady state CSF to plasma water drug ratio and the rate of approach to this ratio were obtained. Striking differences among drugs existed in regard to both parameters. In cancer chemotherapy it is particularly important to know the degree to which chemotherapeutic agents enter CSF. For example, in leukemia CNS involvement is common, yet one of the active drugs, methotrexate, fails to enter CSF. This method was therefore adapted to such patients. Antipyrine, sulfadiazine and *p*-aminohippurate (PAH) were used as standard drugs to which anti-tumor drugs could be compared. The plasma drug concentration was maintained constant and CSF obtained through an indwelling lumbar sub-arachnoid catheter. The steady state CSF to plasma water ratios were similar in man and in the dog, *i.e.*, antipyrine, about unity and sulfadiazine, about 0.8. With PAH neither man nor dog showed significant drug entrance into CSF in eight hours. For antipyrine and sulfadiazine, the time required to reach one-half the steady state ratio in man was much longer than in the dog. This difference may have been accentuated by the different sites of CSF sampling and the larger sub-arachnoid dead space in man. In general, however, the principles established in the dog seem completely applicable to man, and on this basis it is possible to compare the passage of anti-tumor drugs from blood into the fluids of the central nervous system.

Studies of In Vivo and In Vitro Exchange of Erythrocyte and Plasma Phospholipids. CLAUDE F. REED, Rochester, N. Y. (Introduced by Scott N. Swisher).

The structural phospholipids may be important in maintenance of viability of normal, stored and abnormal erythrocytes. *In vivo* incorporation of P^{32} orthophosphate into the phospholipids of plasma, erythrocytes and platelets was measured by serial determinations of specific activity of these lipids in three polycythemic patients. Parallel *in vitro* measurements were made, incubating the patients' labeled plasma and compatible erythrocytes. Previously described chromatographic methods were used to separate the phospholipids.

Erythrocyte lecithin and sphingomyelin appear to exchange with plasma lecithin and sphingomyelin at the rate of 10 per cent per 24 hours. Phosphatidyl serine was not found in cell-free plasma. There was no in-

corporation of radioactivity into erythrocyte phosphatidyl serine in any of the *in vitro* studies. At any point during the first eight days in the *in vivo* studies, the specific activity of erythrocyte phosphatidyl serine was one-half to one-fourth the specific activity of erythrocyte lecithin. The amount of labeling of phosphatidyl serine is thought to measure the contribution of new erythrocyte production to the specific activity of erythrocyte phospholipids.

Normal cells *in vitro* incorporated lipid phosphorous from labeled plasma at a rate equal to that observed *in vivo* for 12 hours. Longer periods of incubation diminished the normal cells' ability to exchange phospholipids, as did prior incubation of the cells in autogenous unlabeled plasma without added glucose.

The erythrocytes of hereditary spherocytosis exchange significantly less lipid phosphorous than normal cells during 12 hours of incubation, with or without prior incubation in autogenous plasma.

These studies demonstrate that the erythrocyte phospholipids, with the exception of phosphatidyl serine, are in a dynamic state of exchange with the plasma phosphatides. It is suggested that these exchanges may be important for the integrity of the red cell, and may be abnormal in certain kinds of intrinsically defective red cells.

The Cardiac Output in Normal Resting Man. JOHN T. REEVES, JAMES E. ROBERTS, ROBERT F. GROVER, GILES F. FILLEY and S. GILBERT BLOUNT, JR.,* Denver, Colo.

Although the use of the direct Fick principle during pulmonary artery catheterization probably provides the most reliable measurement of human cardiac output, a critical analysis of normal values and of relationships existing between the variables of the Fick equation has not been reported. Such analysis of 169 measurements of cardiac output in normal resting man as obtained from this laboratory and from selected published reports revealed a relatively narrow range of arteriovenous oxygen difference (mean, 38.9 ± 6.15 cc. per L.), which is independent of the oxygen uptake. This permitted the definition of a significant linear relationship of cardiac output to oxygen uptake. This relationship accounts, in general, both for the variations in body size and metabolic state of the subjects studied, and it defines more accurately the normal range of cardiac output than does cardiac index.

This study directs attention to the importance of considering the variation between individuals of the arteriovenous oxygen differences in normal resting man. For instance, it is found that the arteriovenous differences of athletes are relatively great and those of men are, in general, greater than those of women. Such findings suggest that the relative amounts of lean and fat mass of the body may be one important factor in the variation of the arteriovenous differences between individuals.

Hemodynamic Relationships of the Anacrotic Slope of the Central Aortic Pressure Pulse in Man at Rest and During Exercise. T. JOSEPH REEVES, LEELA SHOURIE, LLOYD HEFNER and S. H. TAYLOR, Birmingham, Ala. and Birmingham, England. (Introduced by Tinsley R. Harrison).

Pressures in the ascending aorta were recorded at rest and during successively increased levels of uninterrupted exercise in 13 subjects (11 hypertensives, one thyrotoxic and one normal). The slopes of the aortic pressure pulse were continuously recorded by the use of an electronic differentiating circuit, yielding the slopes calibrated in mm. Hg per second. Simultaneous right heart catheterization permitted the measurement of cardiac output, and pulmonary wedge pressure during each level of activity.

The cardiac index varied between 2.37 and 10.8 at rest and between 2.32 and 11.54 during exercise. The O_2 uptake during exercise ranged up to 1,066 ml. per minute per $M.^2$. The maximal slope of the anacrotic limb of the aortic pressure (maximal velocity of pressure rise, referred to hereafter as MvR) ranged from 555 mm. Hg per second to 2,197 mm. Hg per second at rest, and up to 7,300 mm. Hg per second during exercise.

A linear relationship was found between MvR and mean ejection pressure ($r = 0.605$), and similarly between MvR and heart rate ($r = 0.69$). The best correlation, however, existed between MvR and the product of mean ejection pressure, stroke volume and heart rate (minute work) ($r = 0.82$). MvR was plotted against the product of mean ejection pressure and heart rate, and showed a linear relationship for each subject. The separation of the various subjects was consistent with an independent estimate of the status of the myocardium derived by relating stroke volume to pulmonary wedge pressure. Other more complex relationships have been derived that amplify and clarify the above.

The Modification of Myocardial Blood Flow and Oxygen Consumption During Postprandial Lipemia and Heparin-Induced Lipolysis. TIMOTHY J. REGAN, KENAN BINAK, SEYMOUR GORDON, VALENTINO DEFAZIO and HARPER K. HELLEMS,* Detroit, Mich.

The role of the physical state of plasma as a determinant of oxygen availability to tissue may constitute one factor in the induction of myocardial ischemia in man. The effects of plasma alteration during postprandial lipemia upon coronary blood flow (nitrous oxide method) and myocardial oxygen consumption were assessed in seven normal subjects at the time of the maximal plasma lactescence produced by a cream meal of 1.5 Gm. of fat per Kg.

The mean coronary blood flow in a separate group of 15 fasting normals studied as controls, comparable in age and sex, was 81 ± 2.43 ml. per 100 Gm. of left ventricle per minute with a myocardial oxygen extraction of 11.04 ± 0.28 volumes per cent and a myocardial oxygen consumption of 9.02 ± 0.28 ml. per 100 Gm. of left ventricle per minute. In contrast, the mean coronary blood flow during maximal lipemia in the seven normal subjects fed cream was 18 per cent below normal with a value of 67 ml. per 100 Gm. per minute ($p = < 0.001$). The myo-

cardial extraction of oxygen was 10.54 volumes per cent and the calculated myocardial oxygen consumption was 22 per cent below normal with a value of 7.02 ml. per 100 Gm. per minute ($p = < 0.001$). A failure of the usual oxygen extraction increment in the face of a coronary blood flow reduction suggests an impediment to oxygen diffusion during lipemia.

After the administration of 60 mg. of heparin in the seven normal subjects, a 65 per cent decline in plasma lactescence was observed by 45 minutes. At this time, the coronary blood flow, myocardial oxygen extraction and oxygen consumption was 87 ml. per 100 Gm. per minute ($p = < 0.01$), 10.9 volumes per cent and 9.57 ml. per 100 Gm. per minute ($p = < 0.05$), respectively. Thus, the reduced coronary flow and myocardial oxygen consumption were restored in each instance to normal levels by plasma clearing. There were no associated systemic hemodynamic changes to account for these increments. These heparin effects appear dependent on its lipemia clearing property, as no alteration in coronary dynamics was found in four additional patients in whom no change in lactescence took place after the same amount of heparin.

It would appear, therefore, that normal postprandial lipemia constitutes one of the acute factors capable of limiting coronary blood flow and myocardial oxygen consumption in man and that this situation can be modified by lipolysis.

Endogenous Fixed Acid Production and Its Relation to Renal Acid Excretion. Arnold S. Relman * and Jacob Lemann, Jr., Boston, Mass.

If excretion of fixed acid is entirely urinary, total endogenous acid production in the steady state should equal net urinary excretion of acid ("net acid" = NH_4^+ + titratable acid − HCO_3^-). This has never been demonstrated, partly because all sources of endogenous acid have not been identified and measured.

To clarify this question, balances were performed on subjects fed liquid diets of electrolyte-free low-phosphorus soy protein, corn oil, glucose and neutral salts. Over periods of five to eight days, during which acid-base measurements were constant, it was discovered that urinary inorganic sulfate plus organic acids closely approximated "net acid." In six balances, cumulative discrepancies averaged only ± 3 per cent. Conventional calculations of dietary cation-anion balance fell 17 to 32 per cent short of "net acid."

In one study, feeding $NaHCO_3$ equivalent to the control "net acid" produced a new steady state of mild alkalosis during which "net acid" was approximately zero, but sulfate and organic acid excretion were unchanged. After complete recovery, cumulative reduction in "net acid" equaled total bicarbonate given, and total "net acid" plus the fed alkali equaled sulfate plus organic acids.

In another study "net acid" was transiently increased by administration of neutral phosphate. A slight alkalosis ensued which was corrected after cessation of phosphate by transient reduction in "net acid." At the end, after 18 days, cumulated "net acid" again equaled cumulated sulfate plus organic acids.

Increased endogenous acid, produced by methionine loading, has already been shown to be quantitatively recoverable as "net acid."

The endogenous fixed acid which requires renal excretion largely derives from oxidation of sulfur to sulfate and formation of unmetabolized organic acids. Acid-base balance is quantitatively determined by the relation of these acids to "net acid" excretion.

Carbon Tetrachloride Poisoning, a Mitochondrial Lesion. Edward S. Reynolds and Ralph E. Thiers, Boston, Mass. (Introduced by George W. Thorn).

Carbon tetrachloride poisoning in the rat is accompanied by striking changes in the calcium and potassium contents of the liver mitochondria. As early as two hours following the administration of sublethal doses of this chlorinated hydrocarbon, the calcium content of mitochondria may double, reaching a maximum, 20 times normal, at 16 hours. Conversely, the mitochondrial potassium content decreases to half of normal at 16 hours and to a minimum of about a quarter of the control value at 40 hours. Sodium and magnesium contents are but slightly altered, and the sum of the four major cations remains relatively constant. This abnormal electrolyte shift, restricted at first to mitochondria, is observed in the other subcellular components by 40 hours.

When the changes in mitochondrial potassium and calcium contents exceed certain reproducible quantitative proportions, mitochondria lose the ability to couple phosphorylation with oxidation and to oxidize octanoate, glutamate, succinate and β-hydroxybutyrate. The magnitudes of mitochondrial calcium and potassium concentrations allow prediction of the degree of oxidative function of these subcellular organelles.

The alterations in composition and function observed *in vivo* can be simulated by addition of controlled amounts of carbon tetrachloride and calcium to suspensions of normal mitochondria *in vitro*, where detailed studies of the lesion are easily performed. The relationships between electrolyte composition and function of the mitochondria are identical in both cases.

In vitro, normal mitochondria can be protected against exposure to carbon tetrachloride by simultaneous addition of polyvalent, anionic chelating agents such as citrate, adenosine triphosphate and ethylenediaminetetraacetate.

The electrolyte shift of the mitochondria associated with a loss of oxidative function suggests a mode of approach to the study of the effect of other chlorinated hydrocarbons.

Tests of Phosphate Metabolism in Hyperparathyroidism. Telfer B. Reynolds,* Hilda Lanman and Natalia Tupikova, Los Angeles, Cal.

Tests designed to demonstrate the effect of parathyroid hormone on phosphate (P) metabolism were performed on 13 consecutive patients with surgically confirmed hyperparathyroidism. Serum P was within the normal

range in three of the patients, four hour P clearance in six and per cent tubular reabsorption of P (TRP), calculated from creatinine clearance, in nine.

The maximal rate of tubular reabsorption of P (TmP) during a three hour infusion of neutral isotonic P solution was measured in eight of the nine patients with normal TRP. None of these patients had significant azotemia. The calculated quantity of reabsorbed P failed to reach a constant value in consecutive clearance periods in three of the cases so that only an approximation of TmP could be defined. TmP ranged from 1.3 to 4.7 mg. per minute in the eight patients, with a mean of 3.2 mg. per minute. Expressed per 100 ml. glomerular filtrate (creatinine clearance) TmP ranged from 1.8 to 5.4 (mean, 3.6) mg. per minute. With the exception of the lowest one, these values are within the normal range reported by others. In four patients TmP was measured two months postoperatively. In one instance TmP rose (from 2.5 to 5.3 mg. per minute); in one instance did not change; and in two cases it fell (from 4.7 to 1.8 and from 3.6 to 2.0 mg. per minute).

It was concluded that other factors in addition to parathyroid hormone influence renal phosphate excretion and that, with the exception of the serum P level, the tests of P metabolism described above are of little value in the diagnosis of hyperparathyroidism. TmP was difficult to define, varied over a wide range, and was within normal limits in seven of eight patients in whom TRP was normal. It failed to change in the expected manner postoperatively in three of the four patients tested.

Alterations in the Plasma Transport of Thyroxine in Sick Patients and their Relation to the Abnormality in Graves' Disease. JOHN B. RICHARDS, J. THOMAS DOWLING and SIDNEY H. INGBAR,* Boston, Mass.

It has been suggested that in Graves' disease there exists an abnormality in the thyroid hormone-plasma protein complex. This hypothesis is based largely upon increased uptake of I^{131}-labeled thyroxine and triiodothyronine by cellular systems incubated in thyrotoxic sera.

In untreated thyrotoxic patients, thyroxine-binding by the newly-described thyroxine-binding prealbumin of human serum (TBPA) is usually decreased. However, comparable decreases have also been found in patients with other severe acute or chronic illnesses. Precipitous decreases in binding capacity of TBPA may follow surgery or myocardial infarction, and, in serial studies, have been observed within a few hours after parturition and after administration of typhoid vaccine to normal subjects.

These findings prompted a re-examination of the specificity for Graves' disease of the increased *in vitro* cellular uptake of labeled thyroid hormones. Erythrocyte and rat diaphragm uptakes were performed in sera from normal, thyrotoxic and other types of sick patients. In nonthyrotoxic subjects, uptakes were also measured following enrichment of serum with stable thyroxine to thyrotoxic levels of PBI. A close correlation was found between decreased binding by TBPA and the magnitude of the cellular uptake of thyroxine and triiodothyronine. Thus, in normal serum, uptakes at endogenous PBI levels were low and were little increased by enrichment of the PBI. Uptakes at endogenous PBI levels were moderately increased in sera with diminished TBPA in both thyrotoxic and nonthyrotoxic sick patients. In the latter group, uptakes were even further increased when sera were enriched to thyrotoxic concentrations of PBI. Thus, high cellular uptakes of labeled thyroid hormones were conditioned by the level of PBI, but depended principally upon decreased thyroxine-binding by TBPA. Abnormalities in TBPA and in cellular uptake occur in a variety of nonthyroidal illnesses, and are therefore not specific accompaniments of Graves' disease.

Diffusion Characteristics of Ammonia Gas with Special Reference to Intracellular Acid-Base Relations. EUGENE D. ROBIN, PHILIP A. BROMBERG and CLAUDE E. FORKNER, JR., Boston, Mass. (Introduced by Samuel A. Levine).

A study of diffusion characteristics of free NH_3 has resulted in data relevant to three aspects of ammonium metabolism: *1*) the significance and magnitude of total ammonium (NH_4^+/NH_3) levels in normal blood; *2*) the mechanism of ammonium diffusion into cells; and *3*) the use of the NH_4^+/NH_3 system to investigate intracellular acid-base relations.

Arterial NH_4^+/NH_3 concentrations were raised to approximately 600 μM per L. by the intravenous administration of 0.2 M NH_4 acetate to dogs. At this level, alveolar NH_3 tensions (P_{ANH_3}) averaged 7×10^{-4} mm. Hg. Since NH_3 is 1,500 times as diffusible as CO_2, arterial and alveolar NH_3 tensions were assumed equal and the empirical *in vivo* pKa of this system as measured in nine dogs (16 determinations) averaged 9.13 ± 0.15.

Evidence that blood NH_4^+/NH_3 levels measured in dogs and humans *uninfused* with NH_4 acetate are "true" (physiological) and not artifactual was obtained: *a*) NH_3° was found in expired air (P_{ANH_3} averaging 3×10^{-4} mm. Hg.) The calculated pKa values did not differ significantly from those found in ammonium-treated dogs. *b*) The ratio of blood to CSF NH_4^+/NH_3 concentrations was similar in both noninfused and infused dogs.

If NH_3° represents the diffusion form of the buffer pair then the relative concentrations of NH_4^+/NH_3 in extracellular and intracellular fluid will depend on the relative pK's and pH's of these compartments. Substances which modify the pH of both compartments equally will produce no shift, whereas substances which produce relative pH changes between the two compartments will produce shifts in NH_4^+/NH_3 concentrations. During a constant infusion of NH_4 acetate, respiratory acidosis and alkalosis produced only minor changes in plasma NH_4^+/NH_3 concentrations. HCl infusion produced marked increases and $NaHCO_3$ infusion produced marked decreases in extracellular NH_4^+/NH_3 concentrations. These data indicate that NH_3° is the diffusion form of NH_4^+/NH_3, that the intracellular compartment is freely permeable to CO_2, and that equilibrium with H^+ and HCO_3^- occurs slowly.

Effect of Thyrotropin on Inorganic Iodine of the Dog Thyroid. I. N. ROSENBERG,* J. C. ATHANS and A. BEHAR, Boston, Mass.

The early effects of thyrotropin administration were studied by serial measurements of the plasma concentration of labeled iodine in arterial and thyroid venous blood of the pentobarbital-anesthetized dog. A polyethylene catheter in the inferior thyroid vein permitted continuous venous sampling. Total and trichloroacetic acid (TCA)-precipitable I^{131} in plasma were measured, and the TCA-soluble fraction obtained by difference; the latter was largely iodide, as judged by chloroform solubility after exposure to acid iodide-iodate solution and by its electrophoretic behavior.

Two to six days after the animals had received 100 μc. I^{131} intravenously, the thyroidal venous plasma concentration of inorganic I^{131} was usually slightly less than that of arterial plasma while the venous concentration of PBI^{131} exceeded the arterial. Intravenous injection of potassium perchlorate (100 mg.) produced an immediate increase in the thyroid venous $iodide^{131}$ concentration, which became greater than the arterial value. When thyrotropin (5 to 10 U.S.P. units) was then injected intravenously, a prompt sustained further increase (two- to tenfold) in the venous $iodide^{131}$ concentration occurred, and the PBI^{131} concentration rose. The increased thyroid venous concentrations of both PBI^{131} and inorganic $iodide^{131}$ were apparent within 30 minutes after thyrotropin injection, persisted for at least one hour, and the time-concentration curves of both tended to be parallel. Comparable changes in stable iodine were noted. Thyrotropin administered to animals not pretreated with perchlorate also led to an increase in thyroid venous PBI^{131} and to some increase in $iodide^{131}$ concentration; the latter was considerably increased by subsequent injection of perchlorate.

The results suggest that thyrotropin not only enhances thyroidal secretion of hormonal iodine but also increases the thyroidal content of inorganic iodide, the iodide increment originating within the gland.

Study of Possible Causal Role of Lipoprotein Lipase Deficiency in Nephrotic Hyperlipemia. RAY H. ROSENMAN * and SANFORD O. BYERS, San Francisco, Cal.

Studies were done to evaluate the possible deficiency or inhibitor of lipoprotein lipase, variously suggested as responsible for nephrotic hyperlipemia. Observations were in rats made nephrotic by injecting rabbit anti-rat kidney serum. *1)* Lipoprotein lipase activity of postheparin plasma was determined by its ability to increase light transmission and to release unesterified fatty acids (UFA) when added to coconut oil emulsion substrate suspended in normal rat plasma. Plasma was obtained from groups of nephrotic and control rats 10 minutes or at serial intervals after single or repeated doses of varying amounts of heparin. Whether obtained immediately or at serial later intervals, or after repeated doses of heparin, nephrotic rat plasma exhibited as much lipase activity as did the respective control rat postheparin plasma. *2)* The ability of nephrotic plasma itself to support lipolysis of triglyceride substrates was studied by determining ability of normal rat postheparin plasma to increase light transmission and to release UFA when added to coconut oil substrate suspended in nephrotic plasma, previously centrifugally cleared of its endogenous, hyperlipemic lactescence. Hydrolysis of the triglyceride substrate, as measured by UFA release, occurred normally when postheparin plasma was added to the oil emulsion-nephrotic plasma. However, the nephrotic plasma failed to support the sustained lipemia clearing, measured optically, which occurred when the triglyceride was suspended in normal rat plasma. This defect was entirely normalized by addition of bovine serum albumin to the nephrotic plasma. *3)* Plasma utilization or clearance of heparin was assayed by determining clotting times and by protamine titration of blood obtained from nephrotic and control rats serially bled after heparin injection. No abnormality was demonstrated in the handling of exogenously administered heparin by the nephrotic rat.

The data thus indicate that nephrotic hypertriglyceridemia cannot be ascribed to any deficiency or inhibitor of lipoprotein lipase.

The Effect of Changing Cerebral Blood Flow on the Response Time of the Respiratory Center. JOSEPH C. ROSS, JOHN B. HICKAM * and REGINA FRAYSER, Indianapolis, Ind.

The normal respiratory center is exquisitely sensitive to the arterial pCO_2, which implies that it is richly perfused with blood. A simple technique for measuring the speed at which the respiratory center can respond to an abrupt change in arterial pCO_2 has been devised. It is found that the speed of response can be markedly altered by procedures which change cerebral blood flow.

The technique consists of releasing thigh tourniquets which have occluded circulation to the legs of a normal subject for 15 minutes and recording the ventilatory response. This produces abrupt but brief changes in arterial blood (seven subjects: pCO_2 increased 8 ± 3 mm. Hg; pH decreased $0.05 \pm .02$; per cent O_2 saturation decreased 2.2 ± 2.1). Thirteen recumbent male subjects with a resting ventilation of 6.7 ± 1.5 L. per minute had an abrupt increase to 12.3 ± 4.2 L. per minute at a mean time of 29 ± 10 seconds after tourniquet release. When the 13 subjects breathed oxygen, the interval between tourniquet release and increased breathing was markedly prolonged (49 ± 9 seconds). This experiment was repeated in three subjects during infusion of 0.8 per cent NH_4Cl, a cerebral vasoconstrictor. The response time (control, 36 seconds) was more prolonged with NH_4Cl infusion (65 seconds) than with oxygen (54 seconds), while combining NH_4Cl and oxygen virtually prevented a response. On the contrary, infusion of 3.0 per cent $NaHCO_3$ alone (three subjects) or combined with inhalation of 3.0 per cent CO_2 (three subjects), which produce cerebral vasodilatation, considerably shortened the response time during oxygen breathing (36 seconds

on oxygen-$NaHCO_3$ compared with 49 seconds on oxygen alone).

The magnitude of this effect suggests that the vessels of the respiratory center are much more sensitive to certain vasoactive agents than are the cerebral vessels in general. Normal and abnormal respiratory phenomena associated with a rapidly changing state, such as Cheyne-Stokes breathing, may be determined or largely influenced by this apparent lability of the blood supply of the respiratory center.

The Relationship Between Protein Composition of Serum and of Edema Fluid in Heart Failure. RICHARD S. ROSS and JOHN D. S. HAMMOND, Baltimore, Md. (Introduced by E. Cowles Andrus).

Easy availability of subcutaneous fluid in heart failure offers an opportunity to study capillary permeability and the mechanism of edema formation and resorption. As a result of paper electrophoresis of protein in serum and edema fluid obtained simultaneously in 11 patients, the following conclusions have been drawn: There was no relationship between the absolute concentration (mg. per ml.) of total protein, albumin or globulin in serum and the corresponding concentration in the edema fluid. This suggests that the absolute concentration of protein in the edema fluid depends on the relative rates of reabsorption of water and protein. All fractions of serum protein were present in every specimen of edema. A direct relationship was found between the percentage of each of the protein fractions of the serum and the corresponding one of the edema. The slope of the line relating edema albumin concentration (expressed as a percentage of the total protein) to the corresponding serum albumin concentration was 1.20 ($r = 0.89$, $p < 0.001$). Lines relating the various globulin fractions were less steep. The ratio of edema albumin concentration to serum albumin concentration (edema albumin, mg. per ml., to serum albumin, mg. per ml.) is directly related ($r = 0.97$, $p < 0.001$) to the comparable ratio for total globulin. The line relating these two ratios has a slope of 1.79, reflecting the greater permeability of the capillary wall to the smaller albumin molecule.

Thus, the fractional composition of the edema protein depends on the physical charactersistics of the protein molecules and the capillary membrane. In seven patients, serial samples of fluid were obtained. As the amount of edema decreased, there was an increase in the concentration of protein, but the percentage composition of the total protein as regards albumin and globulin remained approximately the same. Thus, while albumin is filtered out of the edema more readily than globulin, there is no preferential reabsorption.

A Study of Cerebral Hemodynamics and Arteriovenous Differences of Glucose, Lactate and Pyruvate Before and After Eating. GEORGE G. ROWE, GEORGE M. MAXWELL, CESAR A. CASTILLO and CHARLES W. CRUMPTON,* Madison, Wisc.

In order to obtain further information concerning the glucostatic theory of hunger, a series of eight patients who had fasted an average of 14 hours were studied by the nitrous oxide method for determining cerebral blood flow, before and 30 minutes after ingestion of a standard meal. Determinations were made of glucose, lactate and pyruvate in arterial and internal jugular bulb blood in the fasting state and 15, 30, 45 and 60 minutes after the meal. Brain uptake of these carbohydrate substances was calculated.

There were no significant differences in cerebral hemodynamics. As compared with the fasting state, during the rise in arterial glucose content, the amount of glucose extracted by the brain increased slightly and transiently. As the peak concentration of glucose in arterial blood was attained, glucose extraction approached the control value. During the falling arterial level, the glucose extraction tended to decrease temporarily, but there was no overall correlation between the absolute arterial glucose level and the brain extraction of glucose. Lactate went through a similar series of changes as did glucose. Pyruvate was given up by the brain during the fasting state but was taken up postprandially so that the brain pyruvate extraction was correlated positively with the arterial pyruvate level.

The data do not clarify the mechanism of hunger as applied to small centers of the brain whose metabolism may be overshadowed by the rest of the organ. However, the change in the arteriovenous glucose difference across the brain through the physiologic range of blood sugar found in this study is much less than that reported for peripheral tissues, and the arteriovenous glucose difference is much less dependent on the arterial glucose level.

Quantitative Studies of Mouse Polyoma (Parotid Tumor) Virus Infection. WALLACE P. ROWE, Bethesda, Md. (Introduced by Joseph E. Smadel).

Mouse polyoma virus can be studied readily by standard virologic procedures, and consequently provides an exceptional model for study of viral neoplasia in a mammal. An experiment was conducted to follow the course of viral growth in mice before and after appearance of tumors. Newborn mice were inoculated with 10^8 infectious doses, and at intervals various tissues and excretions were titrated for content of virus, hemagglutinin, complement fixing antigen and antibody. The growth curve could be divided into two phases: a peak at seven to 10 days, with titers in excess of 10^9 infectious doses per gram of tissue and a plateau for several months thereafter with titers of 10^6 to 10^7 infectious doses per gram. During the peak phase, tissue suspensions contained hemagglutinin in high titers, kidney suspensions titering as high as 1 to 20,000, and urine often having hemagglutinin titers of 1 to 80 or over. In certain tissues, as well as urine, removal of inhibitor by treatment with receptor destroying enzyme was necessary to reveal hemagglutinin. In the plateau phase, which was after antibody had developed, virus was often more easily de-

tected in high dilutions of tissue suspensions than in the undiluted materials, due to the masking effects of antibody. Certain of the concepts obtained from the study may be directly pertinent to searches for comparable agents of human tumors.

Prepartion of a Lipemia-Producing Fraction From Hog Pituitary Glands. DANIEL RUDMAN, FLOYD SEIDMAN and MARIA B. REID, New York, N. Y. (Introduced by Quentin B. Deming).

A previous report [Rudman, D., and Seidman, F. Proc. Soc. exp. Biol. (N. Y.) 1958, **99**, 146] described the occurrence of lipemia in the rabbit following a single subcutaneous injection of the alkaline extract of hog, beef, sheep or human pituitary glands. Eighteen hours after the injection of an extract of 100 mg. of hog pituitary, the serum lipids of the rabbit show a three- to fivefold increase. The average composition of the increment of lipid, which is transported in the serum largely as chylomicra, is 85 per cent triglyceride, 10 per cent phospholipid and 5 per cent cholesterol. A similar lipemic response occurs in the dog. A single hog anterior lobe contains seven times as much lipemia-producing activity as a single hog posterior lobe. Lipemia-producing activity is absent in nine other hog and beef organs. Unfractionated pituitary extracts exhibit lipemia-producing activity eight times greater than can be accounted for by the presence of any of the six recognized anterior lobe fractions.

Lyophilized hog pituitary glands are extracted with 0.1 N NaOH at 5° C. for 15 minutes. The clarified extract, neutralized with acetic acid, is adsorbed on $Ca_3(PO_4)_2$ gel. The lipemia-producing activity is eluted from the gel with 0.3 M NaH_2PO_4. To the eluate at 0° C., acetone is added to a concentration of 40 per cent. The precipitate is discarded, and acetone added to a concentration of 85 per cent. The precipitate is dissolved in H_2O and by successive additions of $(NH_4)_2SO_4$, the fraction precipitated between 20 and 40 per cent saturation is obtained. This precipitate is dissolved in H_2O, dialyzed and lyophilized. The yield is 12 mg. from each gram of lyophilized hog pituitary glands. A single injection of 5 mg. of this fraction produces a three- to fivefold increase in the serum lipids of the rabbit.

Cystinuria-Like Derangement of Amino Acid Excretion in Patients with Hepatic Cirrhosis Given Arginine Intravenously. MARIAN RUSZKOWSKI, JUAN M. BAERTL and GEORGE J. GABUZDA,* Cleveland, Ohio.

The influence of arginine HCl infused intravenously upon the pattern of amino acids in serum and urine was studied in five patients with hepatic cirrhosis maintained on constant diets in a metabolic ward.

Extracts of serum and urine were analyzed qualitatively for amino acid patterns by circular paper chromatography, and quantitatively by elution from bidimensional paper chromatograms (Whatman No. 3 filter paper, solvent systems: *a*) *n*-butanol-acetone-water-diethylamine, 10:10:5:2; *b*) ethanol-water-diethylamine, 77:23:1). Amino acids were identified by R_f values and specific chemical tests.

During initial six day control periods amino acid patterns of sera and urines were qualitatively and quantitatively comparable from day to day. The patients then received 20 Gm. arginine HCl intravenously daily for six days. During and immediately following each arginine infusion, arginine and ornithine levels increased in the sera, but returned to control values within 24 hours. The daily urinary excretion of arginine, lysine and ornithine increased five- to tenfold, and cystine two- to fivefold. These changes appeared on each day of arginine administration and subsided within two days after the last infusion.

Arginine infused intravenously into patients with hepatic cirrhosis caused: *1*) an increased serum ornithine level, probably related to enhanced urea synthesis; and *2*) a transient amino-aciduria similar in pattern to that noted in patients with cystinuria. This observation of amino-aciduria involving arginine, lysine, ornithine and cystine supports the concept of a common renal reabsorptive pathway for these amino acids.

Stimulation by Growth Hormone of Sulfate Uptake by Cartilage of Alloxan-Diabetic, Hypophysectomized Rats. WILLIAM D. SALMON, JR., Nashville, Tenn. (Introduced by William J. Darby).

A protein (sulfation factor) of serum of normal rats and growth hormone treated, hypophysectomized rats was reported to stimulate *in vitro* S^{35}-sulfate uptake by cartilage of hypophysectomized rats, while growth hormone itself had minimal effect. Since high concentrations of insulin slightly increased sulfate uptake under similar conditions and since increased plasma insulin activity has been reported in animals treated with growth hormone and in human acromegaly, the growth hormone effect on cartilage sulfation has been investigated in hypophysectomized rats with acute insulin deficiency.

Treatment of alloxan-diabetic hypophysectomized rats with growth hormone increased sulfate uptake of costal, nasal and xiphoid cartilage in a buffer-glucose medium to two to three times control levels. The uptake was not significantly different from that of cartilage from nondiabetic hypophysectomized rats treated with growth hormone. Sulfation-promoting activity was found in serum of growth hormone treated diabetic rats, though at lower levels than in similarly treated nondiabetic animals.

In further studies with nondiabetic hypophysectomized rats, both insulin and dialyzed serum of normal rats increased sulfate uptake by costal and xiphoid cartilage in a simple buffer medium. A mixture of 13 essential amino acids was synergistic with both insulin and dialyzed serum in stimulating cartilage sulfation; omission of valine from the mixture inhibited stimulation. Addition of glucose to the medium, with or without amino acids, did not increase the effects of either insulin or dialyzed serum.

In a medium containing serum of hypophysectomized rats, amino acids and glucose, when assayed by sulfation-

promoting effect on costal cartilage of hypophysectomized rats, insulin was less potent than dialyzed serum of normal rats and significantly active only in high concentrations.

These results indicate that, despite similarities in the actions of serum sulfation factor and insulin, stimulation of cartilage sulfate uptake by growth hormone is not mediated by insulin.

The Mechanism of the Glycogenolytic Action of Bacterial Endotoxin. JAY P. SANFORD, JACK A. BARNETT and CORA GOTT, Dallas, Texas. (Introduced by Ralph Tompsett).

Hyperglycemia and depletion of liver and muscle glycogen are the most constant biochemical changes which follow injection of endotoxin. These changes have been assumed to result from a hepatotoxic action of endotoxin. As an alternative hypothesis, it is postulated that these changes are mediated through epinephrine release. Epinephrine is known to produce depletion of hepatic glycogen in fed animals but results in increased hepatic glycogen in starved animals. The present study was designed to test this hypothesis by studying the effect of endotoxin in adrenalectomized and normal rats under various dietary regimens.

Adrenalectomized rats were maintained on either desoxycorticosterone acetate or hydrocortisone and were tube-fed a high glucose-balanced diet. Sublethal quantities of endotoxin were injected and 60 minutes later liver glycogen content was analyzed. The mean hepatic glycogen content of intact controls given saline was 21.4 ± 1.3 mg. per Gm. The mean value for intact animals given endotoxin was 6.5 ± 1.1 mg. per Gm. ($p < 0.01$). The mean hepatic glycogen content of adrenalectomized animals given saline was 21.3 ± 2.1 mg. per Gm. In adrenalectomized animals given endotoxin the mean value remained 17.9 ± 1.9 mg. per Gm. ($p > 0.10$).

Intact rats subjected to 96 hours of starvation were studied. In such rats injected with saline, mean hepatic glycogen content was 1.0 ± 0.3 mg. per Gm. Injection of endotoxin into similarly starved rats resulted in an increase in mean hepatic glycogen to 2.0 ± 0.4 mg. per Gm. ($p = 0.05$).

These studies demonstrate that: *1)* Liver glycogen may rise or fall after endotoxin administration depending upon antecedent diet. *2)* Removal of the adrenal medulla abolishes the glycogenolytic effect of endotoxin. *3)* Changes in liver glycogen following endotoxin parallel those known to follow epinephrine.

These data indicate that endotoxin influences hepatic glycogen stores, not as a result of direct hepatotoxicity but rather as the passive consequence of epinephrine release.

A Change in the Regulation of Serum Cholesterol Induced by Homologous and Heterologous Desoxyribonucleic Acid. J. PHILIP SAVITSKY, New York, N. Y. (Introduced by Lewis Leiter).

The physiologic effects of pure, undenatured desoxyribonucleic acids (DNA) injected into mammalian organisms have not been explored. An accidental observation that the nuclear fraction of tissue homogenates altered the serum lipids *in vivo* led to the following investigation.

DNA was carefully prepared from beef brain, beef liver and rabbit liver (of the same strain as the injected animals) by the methods used for the preparation of the bacterial transformation factors. All precautions were taken to prevent denaturation. A single injection of 1 mg. of this DNA was made into each rabbit of groups of 10 normal rabbits per experiment. Over a period of two to three months, the mean serum cholesterol of each group declined significantly (30 to 35 per cent) below normal control levels. This change in the mean largely reflected the decline (50 to 60 per cent) in cholesterol levels of those animals with high normal values. The extent of variation of the cholesterol levels in normal rabbits under controlled environmental and dietary conditions was strikingly reduced following the DNA injection. The standard deviation and coefficient of variation of the serum cholesterol levels decreased to one-third the normal control values. The decline of the mean cholesterol level and the decreasing variation appeared one month after injection, reached a maximum at three months, and showed no return to control levels at the end of six months. The serum phospholipid and neutral fat showed similar changes, paralleling the cholesterol.

The evidence that the DNA in the preparations is the active material includes: the method of preparation, chemical properties and N to P ratio, absence of demonstrable protein, U.V. absorption spectrum, and the incubation with minute amounts of crystalline desoxyribonuclease which completely destroys the activity.

It appears that an injection of DNA improves regulatory control of the serum cholesterol level in the rabbit.

Electron Microscopy of Drug-Induced Cholestatic Jaundice. FENTON SCHAFFNER and HANS POPPER, New York, N. Y. (Introduced by Alexander B. Gutman).

Liver biopsy specimens from patients with jaundice following administration of various drugs producing intrahepatic cholestasis were examined under the electron microscope and in routine and ultrathin sections under the light microscope to attempt to elucidate the site of the injury. Structural disturbances of some hepatocellular substructures were recognized but neither uniformly nor consistently. These included alteration of mitochondria or vacuolization and disruption of the endoplasmic reticulum reflected in basophilic clumps under the light microscope. Accumulation of particulate debris on the sinusoidal surface of the liver cell was more consistently noted. Projections of the liver cells (microvilli) into the perisinusoidal spaces appeared normal, suggesting undisturbed exchange between liver cells and the blood. Two pigments were seen in liver cells, one apparently lipofuscin and the other presumably bile. The most consistent and uniform change was in the microvilli which normally

project into the bile canaliculi. The microvilli in intrahepatic cholestasis were either absent or reduced in number and deformed. The bile canaliculi varied greatly in size. In very dilated canaliculi dense material was seen which represented bile thrombi. Some thrombi contained periodic acid Schiff-positive material under the light microscope. Similar changes in bile canaliculi were encountered in extrahepatic biliary obstruction except that thus far no narrow bile canaliculi have been seen. In extrahepatic obstruction the alteration of the microvilli is possibly the result of increased intraluminal pressure. The frequently undilated but altered canaliculi in intrahepatic cholestasis reflect the absence of increased pressure. This implicates the canalicular membrane as the primary site of the injury in view of the lack of other uniform alterations in the liver cell. The morphologic picture tentatively suggests damage of the canalicular membrane and formation of altered bile with an increased polysaccharide content. It also raises the possibility of regurgitation from dilated bile canaliculi into deep projections of the perisinusoidal spaces between liver cells.

Syndrome of Muscular Dystrophy with Myoglobinuria: Demonstration of a Glycogenolytic Defect in Muscle. RUDI SCHMID* and ROBERT MAHLER, Boston, Mass.

A man with muscular dystrophy was studied, who for 30 years has suffered from severe muscle cramps followed by transient myoglobinuria and prolonged weakness. Cramps occur usually in the extremities and are always precipitated by moderate muscular exercise of 15 to 30 seconds' duration. On the contrary, a minimal degree of physical exertion, such as slow walking on level ground, is tolerated without limitation.

Following exercise or cramps, the normal rise in blood lactate and pyruvate concentrations did not occur. On the other hand, glucose tolerance and response of blood glucose and nonesterified fatty acids (NEFA) to adrenalin, insulin or glucagon were normal. Muscle glycogen content was 2.5 per cent but *in vitro* muscle homogenate formed only one-fifth as much lactate as control muscles. Addition of human muscle gylcogen did not increase lactate production *in vitro*, but with glucose-1-phosphate as substrate, lactate was formed at a rate comparable to control muscles. Muscle phosphorylase activity was found to be less than 5 per cent of normal and no activation occurred after preincubation with adrenalin or adenylic acid.

These observations suggest an enzymatic defect in glycogenolysis in the muscle, most likely involving the phosphorylase system. This may limit the energy available for muscular contraction to sources other than glycogen, with cramps, necrosis and myoglobinuria occurring, if muscular activity is extended beyond this limit. Increased glucose uptake, resulting from simultaneous infusion of glucose and insulin, prevented cramps and improved muscular performance. The high muscle-glycogen content suggests that the mechanisms for glycogen synthesis may not be impaired, the defect being limited to glycogenolysis.

In addition to McArdle's patient, five others with similar clinical and laboratory features have been observed, but without biochemical studies of muscle tissues. The syndrome is distinguished from other muscular disorders by absence of increased lactate concentration after exercise.

The Dialysance of Exogenous Poisons and Some Common Metabolites in the Twin-Coil Artificial Kidney. GEORGE E. SCHREINER,* JOHN F. MAHER and JULIEN MARC-AURELE, Washington, D. C.

Wolf, Remp, Kiley and Currie (J. clin. Invest. 1951, **30**, 1062) derived an expression for dialysance = flow rate $\times \frac{\text{arterial} - \text{venous concentrate}}{\text{arterial} - \text{bath concentrate}}$ or $D = a \times \frac{A - V}{A - B}$. They measured *in vitro* the dialysance of 17 common metabolic solutes at flow rates of 500 ml. per minute in the rotating drum dialyzer. In preliminary experiments with early model twin-coils, we reported (Trans. Amer. Soc. artif. intern. Organs 1957, **3**, 12) small differences in efficiency accountable to surface area. Systematic data on dialysance-flow rate relationships are lacking for the currently used and larger twin-coil dialyzer.

Similarly recent clinical experience with hemodialysis in the removal of exogenous poisons (Arch. intern. Med. 1958, **102**, 896) demonstrated the need for objective data on the dialysance of poisons to provide rational basis for selection of flow rate, duration of dialysis, and prediction of result in specific poisons.

In this study the dialysance of urea, creatinine, inorganic phosphate and chloride was measured over a selected range of flow rates from 50 to 500 ml. per minute.

At 250 ml. per minute flow rate, the dialysance of urea was 130, chloride 120, creatinine 110 and P 60 ml. per minute.

The dialysance of three commonly encountered poisons, bromide, salicylate and phenobarbital, were measured over the same range of flow.

Bromide was the most dialyzable substance encountered, reaching 150 at 250 ml. per minute flow rate and over 200 at 500 ml. per minute of blood flow.

Salicylate described a shallow curve with 80 ml. per minute at 250 and 100 ml. per minute at 500 ml. per minute flow rate.

Phenobarbital described a flattened curve with only a slight rise beyond 200 ml. per minute flow rate. Dialysance was 75 at 250 ml. per minute and 90 at 500 ml. per minute flow rate.

These data permit quantitative comparison with other apparatus and provide a rational guide for those intending to remove a specific metabolite or poison. High flow rates would be a desideratum in the chemical dialysis of bromide but less critical in phenobarbital poisoning.

The Thermolabile Inhibitor in the Latex Fixation Test. ADALBERT F. SCHUBART, Boston, Mass. (Introduced by Charles L. Short).

In the latex fixation test (Singer and Plotz, 1956) biologically inert polystyrene latex particles are coated with human gamma globulin. Aggregation of these particles occurs as a result of the interaction of gamma globulin with, presumably, various abnormal serum constituents. The rheumatoid factor(s) is the most common agglutinator demonstrated in the latex fixation test, which was designed to have a relatively high specificity for rheumatoid sera.

Recently a thermolabile inhibitor was demonstrated in the latex fixation test creating a prozone phenomenon or total inhibition. The latex fixation test was slightly modified in order to accentuate this inhibition phenomenon. Native and heat inactivated aliquots of 106 sera from patients with definite rheumatoid arthritis were submitted to the latex fixation test. No agglutination occurred in 22 per cent of the native samples, though a strong reaction was observed with the inactivated aliquot. The thermolabile inhibitor was also demonstrated in normal sera fortified with rheumatoid factor.

Further studies were carried out in order to determine the nature of this inhibitor. Normal and rheumatoid sera lost their inhibitory properties after exposure to 56° C. for from four to 15 minutes. The inhibitor was absorbed from normal and rheumatoid sera with an antigen-antibody system. The first and second components of serum complement were separated by splitting rheumatoid and normal sera into midpiece and endpiece. The thermolabile inhibitor was demonstrated in the midpiece containing C'1. The endpiece representing C'2 has no inhibitory properties in this test system. Selective destruction of the third and fourth components of serum complement did not significantly alter the inhibitory activity of the thermolabile inhibitor.

It would thus appear that the thermolabile inhibitor of the latex fixation test has some of the characteristics of the first component of serum complement.

Evidence for a Covalent Attachment of the Antidiuretic Hormone to Its Receptor Site in the Kidney. IRVING L. SCHWARTZ,* CONRAD T. O. FONG, EDWIN A. POPENOE, LAWRENCE SILVER and MARY ANNE SCHOESSLER, Upton, N. Y.

Lysine vasopressin, isolated from natural sources by countercurrent and chromatographic procedures, was exposed to 1.1 curies of tritium (H^3) gas for eight days at room temperature and 500 mm. Hg pressure. After removal of labile H^3, firmly labeled H^3-lysine vasopressin (specific activity, 32 $\mu\mu$c. per mg.) was recovered chromatographically as a biologically-active electrophoretically-homogeneous component.

Rats were hydrated, anesthetized with ethanol, and both kidneys dissected free of all attachments except for the renal artery and vein which were exposed separately. Then 1mμ (4 $\gamma\gamma$) of the tritiated hormone was injected into a cannulated external jugular vein. From five to 10 minutes later, just after onset of maximal antidiuretic activity, both kidneys were perfused via the renal artery with saline containing reagents that bind SH groups reversibly (*p*-chloromercuribenzoate) and irreversibly (N-ethyl maleimide). When the kidneys were bloodless they were ligated, excised, homogenized in saline and centrifuged at 1,500 × G for 15 minutes. The pellet (insoluble membrane protein, fibrous protein, nuclei and possibly a few heavy mitochondria) was washed successively with acetone, ethanol and saline until the washings were free of radioactivity. Then, after exposure of the resuspended sediment to conditions that reduce disulfide bonds but do not affect peptide bonds (L-cysteine in 0.15 M $NaHCO_3$, pH 8), the radioactivity of the medium increased over prereduction levels by more than tenfold. Controls showed no comparable release of radioactivity.

These results are interpreted as tentative evidence that the antidiuretic hormone reacts at its receptor site by S-S interchange, probably forming two mixed hormone-receptor disulfide bridges. Such interaction could account *per se* for the effect of -S-S- containing peptide hormones, such as the vasopressins, oxytocin and insulin on the permeability of cell membranes.

Drug Induced Immunological Tolerance. ROBERT SCHWARTZ, Boston, Mass. (Introduced by William Dameshek).

Although many theories have been proposed to explain the formation of antibodies, the precise mechanisms underlying this phenomenon are still obscure. A purine analogue, 6-mercaptopurine, has been found to be a valuable tool for the study of some of these mechanisms. By injecting 6-mercaptopurine treated rabbits with I-131 labeled human albumin it was found that the disappearance of this antigen from the circulating blood was logarithmic, and analogous to the pattern of disappearance of autologous albumin. The usual immune disappearance phase did not develop, nor did humoral antibody appear. A direct relationship was found between the dose of 6-mercaptopurine and reduction of "immunological efficiency" of animals so treated. About one month after the administration of the initial dose of antigen, those rabbits in which the primary immune response was completely blocked by a 13 day treatment with 6 mercaptopurine were injected with a second dose of I-131 tagged human albumin. At this time no additional 6-mercaptopurine was given. The antigen, however, again disappeared logarithmically and without evidence of antibody formation. Skin tests in these "tolerant" animals were done with human albumin, and these were negative. An unrelated antigen, bovine gamma globulin, was injected into these animals during the course of the second experiment and antibody against bovine gamma globulin, but not against human albumin was promptly formed. It was thus possible to induce a state of at least temporary immunological tolerance with 6-mercaptopurine. To explain these findings we advance the hypothesis that the "recognition unit" of the antibody forming cell is a metabolically active system which undergoes an heredi-

table alteration in the presence of an antigen; if this alteration, presumably possessed by a specific group of cells, is blocked by antimetabolites, or if it is inactive due to immaturity, then further injection of that antigen does not provoke an immune response.

The Physiologic Significance of Renal Bicarbonate Reabsorption During Acute Respiratory Alkalosis. WILLIAM B. SCHWARTZ,* GUY LEMIEUX and ADRIEN FALBRIARD, Boston, Mass.

It has previously been demonstrated that in acute sustained respiratory acidosis there is a curvilinear rise in the rate of bicarbonate reabsorption as plasma bicarbonate concentration is progressively elevated from 24 to 55 mEq. per L. Frank excretion of bicarbonate began at approximately 26 to 30 mEq. per L. The relationship between plasma bicarbonate concentration and the rate of bicarbonate reabsorption was found to take the form $y = ax/(b + x)$, an expression mathematically identical with the Michaelis-Menten equation. The present study was undertaken to compare the kinetics of the reabsorptive process during acute respiratory alkalosis with those of acute respiratory acidosis. Renal reabsorption of bicarbonate was examined in normal dogs during acute sustained reduction in pCO_2 to approximately 20 mm. Hg. Plasma bicarbonate concentration, which had been previously lowered by infusion of HCl, was then elevated from 6 to 46 mEq. per L. by infusion of sodium bicarbonate. Frank excretion of bicarbonate began between 16 and 19 mEq. per L. At all concentrations above this, the rate of reabsorption was essentially constant at approximately 1.9 mEq. per 100 ml. glomerular filtrate.

The present data would suggest that in acute respiratory alkalosis, as in the normal, carbon dioxide tension directly determines a fixed reabsorptive limit which is independent of plasma bicarbonate concentration. This pattern contrasts with that found in acute respiratory acidosis, and with partial inhibition of carbonic anhydrase, where the rate of bicarbonate reabsorption appears to be a curvilinear function of plasma bicarbonate concentration. It has been suggested that under these latter circumstances carbonic anhydrase activity is rate-limiting in the series of reactions leading to bicarbonate reabsorption. It is thus concluded that while in both respiratory acidosis and respiratory alkalosis pCO_2 mediates the change in reabsorptive capacity, the nature of the transport limit at low carbon dioxide tensions differs fundamentally from that found with high tensions.

Hormonal Influence on Iron Absorption. MASAFUMI SEKI, MICHAEL FIELD and THOMAS C. CHALMERS,* Boston, Mass.

The endocrine influence on erythropoiesis in hypophysectomized rats has been well delineated, but studies of iron absorption are rare. Determination of whole body radioactivity after intragastric administration of Fe^{59} is an accurate method of measuring low levels of absorption.

$$\text{Per cent absorption} = \frac{\text{seven day whole body radioactivity}}{\text{one hour whole body radioactivity}} \times 100.$$

The percentage of the absorbed dose present in the total circulating RBC after 20 hours (utilization) gives an estimate of the rate of erythropoiesis.

Twelve normal 200 Gm. rats had a mean absorption of 28.9 ± 7.8 per cent (1 S.D.), and utilization of 52.1 ± 7.9 per cent. Four weeks after hypophysectomy the values were 1.5 ± 0.8 and 16.0 ± 7.3 per cent (13 rats). Eight weeks after operation, absorption was 3.8 ± 2.2 per cent and utilization 43.8 ± 12.8 per cent (nine rats).

Individual replacement doses of adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), growth hormone (GH), cortisone, L-thyroxine, testosterone, or 10 mg. of whole anterior pituitary powder (APP) were given to seven groups of six rats each for 10 days. Only the APP group attained normal levels of absorption (24.6 ± 10.6 per cent) and utilization (58.9 ± 10.7 per cent). ACTH, GH and L-thyroxine caused slight but significant increases in both.

Simultaneous replacement doses of cortisone, testosterone, L-thyroxine and GH also raised absorption and utilization to normal (29.1 ± 6.0 and 54.4 ± 11.6 per cent). Excessive doses of ACTH added to the combined hormones did not further increase the values.

Eight weeks posthypophysectomy, rats treated with APP had a mean absorption of 47.7 ± 12.5 per cent and utilization of 72.3 ± 5.2 per cent. When APP-treated animals were given agents known to suppress iron absorption or erythropoiesis, the results were as follows: parenteral iron, absorption 10.7 ± 4.3 per cent, utilization 65.9 ± 4.6 per cent; RBC transfusions, 10.6 ± 4.5 per cent, and 33.2 ± 7.3 per cent; 80 per cent oxygen, 6.5 ± 2.9 per cent, and 48.5 ± 9.9 per cent.

Gastric juice from normal rats failed to increase absorption when administered with Fe^{59} to hypophysectomized rats.

In conclusion, the depressed iron absorption and RBC utilization which follows hypophysectomy responds to replacement therapy, but regulation of absorption is not mediated through the hypophysis.

Organic Acids in Body Fluids of the Uremic Patient. D. SELIGSON, L. W. BLUEMLE, JR., G. D. WEBSTER, JR. and D. SENESKY, Philadelphia, Pa. (Introduced by W. C. Stadie).

The serum electrolytes in the uremic patient show a distortion of the anionic fraction which is a reflection of the accumulation of phosphate, sulfate and organic acids. In 57 uremic patients the concentration of total organic acids ranged from 6 to 26 mEq. per L. compared to an average of 6.1 ± 2.2 in 24 normal subjects. The organic acids from serum of a uremic patient before and after dialysis and the organic acids in the dialysate were fractioned between water and isobutanol by counter-current distribution. The fractionation was followed by measurements of total acidity, optical density at 260 mμ, fluorescence at 400 mμ when activation was at 300 mμ, paper chromatography and other qualitative and quantitative tests on the fractions and their derivatives. Fractionation patterns show that acids were removed by hemo-

dialysis and appeared in the dialysate and that some dialyzed faster than others but not as fast as urea. The dialysate contained identifiable quantities of acetate. Lactate represented 20 per cent of the total organic acids in normal serum, a lesser fraction in uremic serum, but the largest component in the dialysate. Other acids such as citric and malic represented insignificant amounts. Fluorescent peaks, distribution coefficients, qualitative tests and paper chromatography demonstrated 5-hydroxyindoleacetic, other indoles, conjugated phenols, hippuric, itaconic and several other acids. The uremic odor appeared to concentrate in one fraction. The more polar fractions injected into mice appeared to quiet them, while less polar fractions appeared to stimulate them. In summary, many organic acids differing in quality and quantity from those in normal serum appear in the serum of uremic patients and are removable in part by dialysis. These may have pharmacologic properties which contribute to the signs and symptoms of the uremic syndrome.

Metabolic Intermediates of the Rabbit Red Blood Cell during Storage in Acid-Citrate-Dextrose (ACD) and in ACD-Inosine. A. WILLIAM SHAFER and GRANT R. BARTLETT, La Jolla, Cal. (Introduced by E. L. Keeney).

The viability of rabbit or human red cells appears to be related to their content of a large pool of water-soluble organic phosphate, which, during storage, decreases more rapidly in ACD than with added adenosine or inosine. To examine the nature and changes of the phosphate compounds during storage, rabbit blood was incubated at 4° in ACD and in ACD-inosine and the red cells were analyzed by ion-exchange chromatography at 0, 21 and 42 days.

The following (in micromoles P per ml. RBC) accounted for most of the phosphate in ACD and ACDI at 0, 21 and 42 days: total-P (ACD-29.2, 24.4, 21.1) (ACDI-29.0, 27.9, 24.5); inorganic-P (ACD-0.7, 10.8, 14.4) (ACDI-0.5, 6.9, 8.4); 3-diphosphoglycerate (ACD-21, 5.6, 1.2) (ACDI-21, 9.3, 3.9); adenosine triphosphate (ATP) (ACD-4.0, 1.2, 0.3) (ACDI-4.4, 1.4, 0.5); sedoheptulose-7-P (ACD-0, 0.7 0.3) (ACDI-0.1, 3.1, 3.6); adenosine diphosphate (ADP) (ACD-0.7, 0.5, 0.4) (ACDI-0.7, 0.7, 0.9); glucosediphosphate (0.6 to 0.7, all assays); fructosediphosphate (< 0.02, all assays).

Although there was extensive conversion of organic-P to inorganic-P (significantly less with inosine) there were striking differences in the behavior of the individual compounds in both storage media. The inosine had little influence on the breakdown of ATP but slowed the loss of diphosphoglycerate considerably. It had been expected that the ribose portion of inosine would be metabolized but it was surprising to find that, of all the possible pentose-shunt intermediates, only sedoheptulose-7-phosphate accumulated in significant amount. Of interest was the rapid disappearance of fructosediphosphate on mixing the blood with ACD and the stability of glucosediphosphate throughout storage (the normal rabbit red cell ratio for fructosediphosphate to glucosediphosphate is 4 to 1).

Aside from the significance to blood banking, the results further emphasize the increasing usefulness of the red cell as a tool for studies of carbohydrate metabolism.

Studies on the Homeostatic Control of Cholesterol Synthesis. MARVIN D. SIPERSTEIN * and M. JOANNE GUEST, Dallas, Texas.

Despite variations in dietary cholesterol, tissue cholesterol levels vary relatively little. This is in part due to the fact that increased cholesterol intake results in depressed cholesterolgenesis, while decreased dietary cholesterol stimulates this process. The mechanism by which this feed-back control of cholesterol synthesis operates is poorly understood. Studies have therefore been performed to determine: *1*) where in the biochemical steps of cholesterol synthesis this homeostatic regulation is exerted; and *2*) how this control may be mediated.

The reactions of cholesterol synthesis are as follows: acetate---1--→ acetyl-CoA---2--→ acetoacetyl-CoA---3--→ β-hydroxy-β-methylglutaryl-CoA (HMG-CoA)---4--→ mevalonic acid--→-5-→---→ squalene--→6-→--→ cholesterol. Rat liver slices were incubated with acetate-C^{14}, mevalonate-C^{14} and squalene-C^{14}, and the influence of 2.5 per cent cholesterol diets on the conversion of these substrates to cholesterol was determined. Cholesterol feeding depressed cholesterol synthesis from acetate 12- to over 100-fold; however the conversion of squalene to cholesterol was not affected and cholesterol synthesis from mevalonate was decreased only threefold. Reactions 5 and 6 can therefore be eliminated as important sites of the feed-back inhibition of cholesterolgenesis. Acetoacetyl-CoA is an intermediate in fatty acid synthesis and HMG-CoA is required for the formation of ketone bodies; therefore Reactions 1 and 2 can be excluded as sites of the cholesterol inhibition by the fact that cholesterol feeding did not effect lipogenesis from acetate. Likewise Reaction 3 is intact since ketone body synthesis was not inhibited.

It is concluded therefore that Reaction 4, involving the conversion of β-hydroxy-β-methylglutaryl-CoA to mevalonate, must represent the site of the physiological feed-back control of cholesterol synthesis. Finally, cholesterol, cholestryl-palmitate, -oleate, -linoleate and -arachidonate can probably be excluded as direct mediators of this homeostatic control since their addition *in vitro* will not significantly depress cholesterolgenesis.

Effects of Spirolactones on Excretion of Water and Electrolytes, and on Aldosterone Metabolism in Cirrhosis. MARVIN H. SLEISENGER,* JACK RICHARD, O. DHODANAND KOWLESSAR, BAYARD CLARKSON, DAVID THOMPSON and RALPH E. PETERSON,* New York, N. Y.

Following control observations of from one to six months in the hospital, two patients with fluid retention due to cirrhosis received one of two spirolactones, SC 9420 or SC 8109. The former compound was adminis-

tered orally while the latter was given both orally and intramuscularly in daily doses of 400 mg. and 250 to 1,000 mg., respectively. Two treatment periods of 10 days each in one individual (SC 9420) and periods of 26 and 12 days in the other (SC 8109) resulted in diuresis of 30 and 46 lbs., respectively. These subjects, with marked ascites and edema, had become refractory to conventional therapy which included mercurials, chlorothiazide and acidifying agents both individually and in combination.

Although urinary sodium excretion increased significantly within 24 hours of administration of these drugs, diuresis did not commence until the fourth to fifth day. Sodium output rose steadily from 3.0 to 240.0 mEq. per 24 hours (SC 9420) and from 20.0 to 190.0 mEq. per 24 hours (SC 8109). Both natriuretic and diuretic effects persisted for three to four days after withdrawal of the agents, the 24 hour urinary sodium gradually falling to 3.0 and 6.0 mEq., respectively. Within a narrow range the daily sodium intake had no influence upon the results, being 51.3 mEq. in one subject and 17.1 in the other. In one patient there was a fourfold increase in sodium to potassium ratio in the saliva during spirolactone therapy.

Urinary aldosterone measurements were made prior to and during the course of treatment with the spirolactones. In addition, tritium-labeled aldosterone was used to measure the rate of turnover of aldosterone.

Pyrimidine Studies in Pernicious Anemia. LLOYD H. SMITH, JR. and FAITH BAKER, Boston, Mass. (Introduced by Edward Bland).

Methods have been recently developed to measure quantitatively in circulating human blood cells the activities of three enzymes involved in pyrimidine synthesis: aspartate carbamyltransferase (ACT), dihydroorotase (DHO) and dihydroorotic dehydrogenase (DHO-D). Hemolysates of normal erythrocytes and sonicates of normal leukocytes have shown reproducible levels of these enzymatic activities per cell. In seven patients with untreated Addisonian pernicious anemia ACT and DHO were found to be 3 × increased per erythrocyte and 2 × increased per leukocyte. The DHO-D activity was normal in pernicious anemia leukocytes and absent (normal) in pernicious anemia erythrocytes. In the leukocyte this pattern of activity is in contrast to that found in immaturity of cells *per se* (myelocytic leukemia, myeloid metaplasia) where the increase of DHO-D activity is usually the most striking abnormality. Erythrocyte enzyme levels in pernicious anemia are as high as those found in Di Guglielmo's syndrome. The abnormal enzymatic pattern of pyrimidine synthesis reverts to normal following B_{12} therapy.

It is suggested that these increased enzymatic activities may be secondary to a partial block at a later stage in pyrimidine nucleotide formation during B_{12} deficiency with a compensatory "negative feedback" hypertrophy of the earlier enzymes. The enzyme pattern found in pernicious anemia blood cells resembles that which has been described in "pyrimidine starvation" of bacteria with genetic blocks in the pyrimidine synthetic pathway (Yates-Pardee). Indirect evidence for such a control mechanism for pyrimidine synthesis in man has been previously noted in orotic aciduria, where nucleotide therapy markedly reduced the urinary excretion of orotic acid. A second feedback control mechanism described in bacteria, the competitive inhibition of ACT by cytidine-5-phosphate (endproduct inhibition of the first biosynthetic step) was found to be absent in human leukocytes and erythrocytes.

Inhibition of Growth Hormone Activity with Modified Growth Hormone Preparations. MARTIN SONENBERG * and WILLIAM L. MONEY, New York, N. Y.

The treatment of beef growth hormone with acetic anhydride results in inactivation of the growth hormone. This acetylated growth hormone is capable of inhibiting the growth promoting effects of unmodified growth hormone in hypophysectomized rats. Growth hormone preparations treated with other reagents as well as bovine serum albumin treated with acetic anhydride failed to inhibit the growth response of hypophysectomized rats to unmodified growth hormone.

Such acetylated preparations of growth hormone are also capable of inhibiting several biochemical changes produced by active growth hormone. The increase in serum phosphorus and alkaline phosphatase produced by the administration of active growth hormone was not observed when acetylated growth hormone was administered with the active hormone preparation. Furthermore the administration of acetylated growth hormone alone caused a fall in serum inorganic phosphorus and alkaline phosphatase in intact rats.

It has also been demonstrated that the hypoglycemic response of a single injection of growth hormone and the hyperglycemic response of chronic administration of growth hormone were modified by the simultaneous administration of acetylated growth hormone preparations.

On the Nature and Properties of Blood Thromboplastin. THEODORE H. SPAET * and JOSE CINTRON, New York, N. Y.

Bergsagel and Hougie obtained a preparation with thromboplastic activity from washed platelets which had been preincubated in a system containing adsorbed plasma, serum and calcium ion. In the present study similar activity was sedimented with crude phosphatide suspension.

$BaSO_4$-adsorbed, oxalated plasma was incubated for 15 minutes with serum and Ca^{++}. Fibrin was removed, a "cephalin" suspension was added, and incubation continued for an additional 15 minutes. The mixture was centrifuged for 30 minutes at 18,000 rpm, and the resultant sediment resuspended in Ca^{++}-containing solution. The sediment was centrifuged and resuspended two additional times as before. The final suspension was diluted such that it clotted normal human recalcified plasma in 12 seconds. Clotting was not produced in a Ca^{++}-free system,

showing an absence of thrombin. Identical clotting times were obtained with normal, factor V-deficient and Stuart factor-deficient plasmas. Activity was stable at 37° C. for one hour, but gradually disappeared thereafter. Activity was lost at 4° C. if the $BaSO_4$ plasma was of human origin, but was stable if bovine reagent was used. It was unstable on freezing or heating to 56° C. for 10 minutes. Incubation with serum resulted in inactivation, and activity could not be recovered by subsequent sedimentation and washing. Activity was lost by washing the sediment in solutions free of Ca^{++}. Incubation of the "cephalin" suspension with either plasma or serum reagent separately in the presence of Ca^{++} failed to produce additional clotting activity in the washed sediment over that found in the unincubated cephalin alone.

The preparation described above has the properties previously attributed to blood thromboplastin. It would appear to be a lipid-protein complex, the stability of which is maintained by calcium ion.

The Hemodynamic Effects of Methoxamine in Mitral Valve Disease. C. Alpheus Stanfield, Peter Perkins, Herbert Constantine, Milton Luria, James Finlayson, Frank W. Lovejoy, Jr. and Paul N. Yu, Rochester, N. Y. (Introduced by Nolan L. Kaltreider).

The effects of intravenous methoxamine (Vasoxyl®) in subjects with mitral valve disease and no cardiovascular abnormality were studied by right heart catheterization and isotope dilution technique.

No consistent change occurred in oxygen consumption, arterial oxygen saturation, effective stroke volume or pulmonary vascular resistance.

Effective cardiac output decreased and arteriovenous oxygen difference increased in most subjects. Mitral valve flow decreased in all subjects with mitral stenosis.

Total systemic and "left heart" resistances were usually elevated.

Right ventricular end-diastolic pressure and stroke work were unchanged or slightly increased.

Significant increase in pulmonary "capillary" ("PC") pressure and its "V" peak was usually observed in patients with mitral insufficiency, and combined mitral valve disease, but not in those with "pure" mitral stenosis or no cardiovascular abnormality.

There was a delay in all time components of isotope dilution curves during methoxamine infusion. Disproportionate prolongation of build-up and disappearance times was characteristic of mitral insufficiency. The "central blood volume" usually remained unaltered.

It is concluded that methoxamine produces constriction of systemic arterioles and probably systemic venules, with elevation of systemic arterial pressure and reduced rate of blood flow.

Increased left ventriculo-atrial pressure gradients during methoxamine infusion were usually balanced by a shortened systolic ejection period so that mean mitral regurgitant flow probably changed little in patients with mitral insufficiency. Mitral regurgitant stroke volume was estimated usually to be increased, however.

In patients with mitral insufficiency the increase in "PC" pressure and its "V" peak is probably due to augmented mitral regurgitant stroke volume and/or left ventricular decompensation. When the ratio of the increment in "V" peak pressure to that of systemic arterial systolic pressure $\left(\frac{\text{increase "V"}}{\text{increase FA}_s} \times 100\right)$ exceeded 20 per cent, significant mitral insufficiency usually was present. This study may thus serve as a useful means of detecting the presence of mitral insufficiency.

Antigenicity of Human Vascular Endothelium and Its Relationship to the Pathogenesis of Vasculitis. Mario Stefanini,* Sergio Piomelli and Rose H. Mele, Boston, Mass.

Two major groups of antibodies developed in rabbits receiving human vascular endothelium extracts. One group could be removed by absorption with human serum. By precipitation, complement fixation and so forth the other group of antibodies reacted against the specific endothelial antigens and extracts from kidney and spleen but not from liver. By use of a fluorescein tagging technique, the anti-endothelial antibody was localized at the level of the endothelial layer of vascular intima. Antisera could also be prepared against the adventitia, but not against media from human aorta. Vascular antigens from whole aorta induced formation of specific antibodies against adventitia and intima constituents only. Antigens proper to the vascular wall appeared unrelated to platelet antigens and showed no organ specificity. By virtue of localization, they might represent a substance homologous to Forssman's antigens in Forssman's negative species.

Experiments were conducted *in vivo* to elucidate the relationship of the antigenicity of vascular constituents and pathogenesis of vasculitis. No hemorrhage nor vasculitis occurred in man or animals after injection of heterologous anti-endothelial serum, local (man) or parenteral. Anaphylactoid shock in guinea pigs and rabbits and delayed nephritis in dogs followed instead. A precipitin reaction between the serum from patients with periarteritis nodosa (eight cases), anaphylactoid purpura (nine) and lupus erythematosus (12) and aorta extracts of human origin was shown to be nonspecific by analysis with double diffusion agar precipitation method. Reaction appeared due to a serum factor reacting with the gamma globulin which contaminates most vascular antigens.

In conclusion: *1)* Adventitia and intima of vessels are antigenic, certainly hetero-antigenic. *2)* Vasculitis in man may develop in the course of a specific, but not autospecific, antigen-antibody reaction. As such, it is not a true autoimmune disorder.

I^{131}-Albumin Turnover Studies in Cushing's Syndrome. Kenneth Sterling,* New York, N. Y.

Several features of Cushing's syndrome are regarded as manifestations of tissue protein wasting, *e.g.*, osteo-

porosis, muscle atrophy, thin skin and diminution of fibrous tissue repair. To investigate the metabolism of one important protein, I^{131}-labeled albumin turnover studies were carried out. Three cases of Cushing's syndrome due to bilateral adrenal hyperplasia were studied with simultaneous normal subjects, by the method previously reported.

The cases of Cushing's syndrome revealed significantly accelerated albumin turnover (mean biologic half-time of 9.6 days, as compared with the mean half-time of 14.1 ± S.D. 1.4 days in the control group). The most advanced case with osteoporosis and collapsed lumbar vertebrae exhibited pronounced reduction of the total exchangeable albumin pool (178 Gm., as compared with the mean value of 302 ± S.D. 28 Gm. in the control group). This patient had the fastest albumin turnover (half-time, 7.7 days). During the months following bilateral adrenalectomy gradual clinical improvement occurred with resumption of menstruation. In studies 11 and 15 months after adrenalectomy the turnover rate was slower (half-time, 10.0 days) but had not reverted to normal.

The data are compatible with other investigators' findings of accelerated albumin turnover during the administration of cortisone or hydrocortisone to normal subjects. Similar kinetic data have been obtained in active thyrotoxicosis. The interpretation offered is that in severe Cushing's syndrome, accelerated degradation leads to reduction of the body albumin pool, after which a new equilibrium is established with a smaller pool.

The gross similarity in the effects upon protein metabolism produced by the adrenocortical and thyroid hormones raises the question of whether similar or different mechanisms are involved. The precise mode of hormonal regulation remains to be elucidated.

Detection of the Heterozygous Carrier of the Wilson's Disease Gene. IRMIN STERNLIEB, ANATOL G. MORELL and I. HERBERT SCHEINBERG,* New York, N. Y.

Parents of patients with Wilson's disease, which is probably transmitted in autosomal recessive fashion, are asymptomatic but are presumably heterozygous for the abnormal gene. A reduced concentration of the serum copper-protein, ceruloplasmin, is characteristic of Wilson's disease and is sometimes observed in parents, but this finding is not regular enough to indicate the heterozygous state reliably. We have found an abnormality in heterozygotes which appears to distinguish them quite sharply from control subjects.

After ingestion of cupric^{64} acetate a peak in the curve of serum concentration of copper^{64} appears in one or two hours in almost everybody. There follow a prompt fall and a slow secondary rise in the curve. The first peak represents nonceruloplasmin serum copper; the secondary rise, which varies considerably in height in different individuals, is due to copper^{64} which has been incorporated into serum ceruloplasmin. Comparing the height of the secondary rise to the initial peak relates the degree of this incorporation to the copper^{64} absorbed.

In a group of 16 control subjects, with a dose of 2.0 mg. of copper^{64}, the ratio of the serum copper^{64} concentration at 48 hours to the concentration of the initial peak ranged from 0.822 to 2.983, with a mean of 1.518 and a standard deviation of 0.21. In contrast, in a group of 10 parents of patients with Wilson's disease, fed the same dose of copper^{64}, this ratio ranged from 0.100 to 0.857, with a mean of 0.515 and a standard deviation of 0.22. Only one ratio from each group fell within the range of the other group. Eight of the parents had normal ceruloplasmin concentrations.

The relatively low incorporation of copper^{64} into ceruloplasmin in heterozygotes may reflect a larger than normal copper pool with which copper^{64} readily mixes, or an aberration in synthesis or metabolism of ceruloplasmin.

The Effects of Total Body Irradiation on the Production of Antibody in Man. IRWIN L. STOLOFF, FARID HAURANI and EVALYN REPPLINGER, Philadelphia, Pa. (Introduced by W. Paul Havens, Jr.).

The treatment of human leukemia with roentgen radiation of the whole body followed by transplantation of homologous marrow furnished an opportunity to determine: *a*) whether patients receiving varying dosages of radiation could produce antibody, and *b*) whether infused homologous marrow could produce another antibody in its new environment. Six patients with acute leukemia and one patient with disseminated neuroblastoma were given single doses of roentgen radiation ranging from 170 to 800 r. (midplane of the body). Immediately after, these patients received infusions of homologous marrow (5 to 20 billion cells) from donors who had shortly before been given a "booster" dose of tetanus toxoid. Four to six days after radiation, the patients (Schick-negative) were given a "booster" dose of diphtheria toxoid. In five patients who received 170 to 490 r., the immunologic response was vigorous, ranging from 18 to 150 units diphtheria antitoxin per ml. serum. Qualitatively, the pattern was similar to that found in normal persons, with a significant increase in antibody by the seventh day after inoculation, reaching a maximum in 14 to 28 days. The two patients who received 800 r. had no increase in their circulating diphtheria antitoxin, although in one patient this was measured only on the seventh day (time of death) after injection of toxoid, and in the other on the seventh and 14th day (time of death) after injection. There was no apparent relationship between the immunologic response and lymphopenia or neutropenia that developed, and large amounts of antitoxin were produced during periods when circulating lymphocytes and neutrocytes numbered less than 50 cells per $mm.^3$ of blood.

The attempts to demonstrate the production of antibody (tetanus antitoxin) by the infused marrow in its new environment (irradiated patients) were unsuccessful. Six of these patients received cells from donors who produced 12.5 to 102 units tetanus antitoxin per ml. serum, but none of the six recipients had any significant increase in circulating tetanus antitoxin.

An Abnormality of Nonesterified Fatty Acid Metabolism in Alcoholic Patients with Cirrhosis. JAMES M. STORMONT and JOSEPH E. MACKIE, Boston, Mass. (Introduced by Charles S. Davidson).

Plasma nonesterified fatty acids (NEFA) are considered an important form of fat transport to tissues for oxidative metabolism. We have observed abnormal plasma NEFA concentrations (method of Dole) after fasting and insulin hypoglycemia in patients with cirrhosis.

Random plasma NEFA concentrations following overnight fast were: 22 controls, 768 μEq. per L. ± 42 S.E.; 17 chronic cirrhosis, 898 μEq. per L. ± 47 S.E.; 13 cirrhosis with coma or hepatocellular failure, 1,269 μEq. per L. ± 126 S.E. ($p < 0.01$).

In a group of five control subjects and 10 cirrhosis patients insulin (0.1 unit per Kg.) was given following 16 hours' fast. The cirrhosis patients had a delayed rise in blood glucose, so that at 90 minutes mean concentrations were 65 mg. per cent ± 3.1 S.E. (74 per cent of fasting) compared to 78 mg. per cent ± 1.5 S.E. (95 per cent of fasting) in controls ($p < 0.02$). Mean fasting NEFA concentrations were higher in the cirrhosis patients than in the controls (1,196 μEq. per L. ± 133 S.E. *vs.* 958 μEq. per L. ± 118 S.E.), falling 45 minutes after insulin to 53 per cent of fasting in cirrhosis, 43 per cent in controls. Ninety minutes after insulin when the blood sugars of many cirrhosis patients had not returned to normal, their NEFA concentrations had risen at least to fasting values, but not the controls: cirrhosis, 1,171 ± 113 S.E., 98 per cent of fasting; controls, 524 ± 120 S.E., 55 per cent of fasting ($p < 0.01$).

Three patients with severe hepatocellular failure had a greater abnormality with a fall to 22 per cent of fasting values 45 minutes after insulin and rise at 90 minutes to 127 per cent of fasting. One of these patients studied after one month of clinical improvement on an adequate diet had a normal response to fasting, but the abnormal response to insulin remained. Liver biopsy showed fat still present.

Clinical estimations of subcutaneous fat, of serum albumin, blood ketones and lactic acid concentrations did not correlate with the NEFA response.

It is not known whether the increased NEFA concentrations in cirrhosis patients after fasting and insulin are due to increased peripheral production or to decreased oxidation or removal.

Pulmonary Vascular Resistance Following Closure of the Atrial Septal Defect in Patients with Severe Pulmonary Hypertension. H. J. C. SWAN, WALTER BECK and HOWARD B. BURCHELL, Rochester, Minn. (Introduced by Ward S. Fowler).

Ten patients with pulmonary artery systolic pressure in excess of 60 mm. of mercury were studied before and four to 24 months after complete closure of their atrial septal defects. Reductions in pulmonary vascular resistance of unexpectedly large magnitude were observed in some of the patients. The following averages obtained before operation: pulmonary artery systolic pressure, 85 mm. of mercury (range, 66 to 109 mm.); atrial pressure, 6 mm. of mercury (2 to 8 mm.); pulmonary blood flow, 4.5 L. per minute per $M.^2$ (2.3 to 7.1 L.), and pulmonary vascular resistance ($R_{pv} = [\bar{P}_{pa} - \bar{P}_{la}] \div Q_{pa}$), 560 dyne-sec-cm.$^{-5}$ (200 to 1,000). After operation significant reductions were observed in pulmonary artery systolic pressure (average reduction, 45 mm. of mercury) and in pulmonary blood flow (average reduction, 1.6 L. per minute per $M.^2$). The average pulmonary artery wedge pressure was 12 mm. of mercury after operation. R_{pv} increased from 760 to 850 dyne-sec-cm.$^{-5}$ in one patient, but declined by 85, 75, 70 and 40 per cent in four other patients with preoperative values of more than 600 dynes. The decline in the remaining patients in whom R_{pv} was not greatly increased averaged 30 per cent. The level of R_{pv} after operation was significantly less than the minimal value attained preoperatively during the breathing of 100 per cent oxygen, and in one case during infusion of acetylcholine.

The reduction of R_{pv} to values close to or within the range of normal subsequent to closure of atrial septal defects in certain patients with severe pulmonary hypertension suggests that resolution of organic pulmonary disease has occurred, or that a reversible vasoconstrictor factor plays a more significant part in the determination of R_{pv} than was thought to be true heretofore.

Selective Inhibition of Poliovirus Multiplication. IGOR TAMM * and MARJORIE M. NEMES, New York, N. Y.

Strong evidence has accumulated that virus synthesis in host cells is initiated and directed by nucleic acid from the infecting virus particle. It appears that host cells contribute precursor materials, enzymes and energy to virus synthesis. This process takes place in the host cell protoplasm in the temporary absence of a limiting virus membrane. Metabolic inhibitors which interfere with the synthesis or utilization of precursors common to host and virus or with cellular energy yielding mechanisms inhibit virus multiplication, but may be damaging for host cells. It seems probable, however, that quantitative differences exist in biosynthetic conditions, requirements and rates which may render virus multiplication more susceptible than the host cell to the effects of certain inhibitory compounds.

Earlier work with benzimidazole derivatives in this laboratory revealed some differences in virus selectivity of compounds. More striking evidence of selectivity has been obtained recently with 2-(α-hydroxybenzyl)-benzimidazole. At a concentration of 0.11 mg. per ml. this compound caused 99 per cent inhibition of Type 2 poliovirus multiplication in monkey kidney cells but showed no inhibitory activity against influenza B virus either in these cells or in the chorioallantoic membrane. At the 99 per cent inhibitory concentration this compound produced only minimal microscopic changes in monkey kidney cells but it markedly delayed the development of cytopathic effects due to poliovirus. The curves relating

inhibition of virus multiplication or reduction in virus induced damage to concentration of the compound were parallel. The cell protective effect varied directly with concentration of compound and inversely with size of virus inoculum. 2-(α-Hydroxybenzyl)-benzimidazole not only failed to inhibit influenza virus multiplication in monkey kidney cells but also failed to protect such cells against the cytopathic effect of this virus. It should be emphasized that the compound is virostatic, but not virocidal, for poliovirus.

The Characteristic Pattern of Esophageal Dysfunction Due to Hiatal Hernia Demonstrated by Fluorocinematography and Simultaneous Pressure Recording. E. Clinton Texter, Jr., Harold P. Lazar, Ernesto J. Puletti and Gaston Vantrappen, Chicago, Ill. and Louvain, Belgium. (Introduced by David P. Earle).

The anatomic derangements accompanying hiatal hernia provide an experimental situation wherein the contributions of the intrinsic (physiologic sphincter) and extrinsic (diaphragm and esophago-gastric angle) components of the closing mechanism at the gastro-esophageal junction can be studied. Contraction, relaxation and propulsion of the bolus were recorded by an X-ray television system (GE-TVX) and a synchronized movie camera. Intraluminal pressures were measured using fluid filled catheters connected to external transducers and a four channel amplifier-recorder. Correlation of the fluorocinematographic appearance with the intraluminal pressure waves was achieved by superimposing the pressure tracings on the cine film. The findings obtained in 65 patients with various types of hiatal hernia were compared to the patterns from normal subjects.

Two abnormalities of the esophagus were found to be characteristic of hiatal hernia: *1*) Dysfunction of the esophago-gastric closing mechanism. The normal "high pressure zone" between the gastric fundus and the esophagus was usually displaced upward in patients with sliding hiatal hernia. The pressures in this zone, which normally exceed fundic pressure, were frequently equal to those recorded from the herniated stomach, thus imposing no barrier to gastro-esophageal reflux. The upward displacement of the gastro-esophageal vestibule could be recognized between the hernia and the esophagus. Failure of relaxation of the physiologic sphincter in response to swallowing was frequent. *2*) Abnormal motor activity of the esophagus. This consisted of abnormal deglutition complexes in the terminal 10 cm. of the esophagus and frequent, rhythmic, nonperistaltic contractions whose amplitude exceeded that of the primary peristaltic wave.

This represents the most accurate technique for the diagnosis of hiatal hernia. The observations confirm the presence of an intrinsic lower esophageal sphincter which is less efficient when under the influence of negative intrathoracic pressure. Dysphagia has been correlated with abnormal deglutition gradient; heart burn and substernal pain, with nonperistaltic motor activity.

Treatment of Acute Leukemia by Supra-Lethal Whole-Body Irradiation and Isologous Marrow Transplantation. E. Donnall Thomas,* Harry L. Lochte, Jr. and Otto D. Sahler, Cooperstown, N. Y.

Leukemia in one of a pair of identical twins has been studied in two sets of twins. A girl, aged four, was in relapse despite conventional therapy. She received an air dose of 850 r. of uniform radiation over 27 hours from two symmetrically placed Co^{60} sources. Then 3.9×10^9 nucleated marrow cells from her normal twin were infused intravenously. No radiation sickness was observed. Leukocytes, platelets and reticulocytes declined to minimal values in eight days. Recovery of all three formed elements began about the thirteenth day and was complete by the thirtieth day. Remission continues after 67 days.

A female, aged three, in relapse received a tissue dose of 250 r. from a 250 KV. X-ray machine, followed by 1.27×10^9 nucleated marrow cells from her twin. This was followed by depression of formed elements and a subsequent return of normal cells. Leukemia recurred after two months. Three months after the first irradiation the patient received an air dose of 1,140 r. of Co^{60} radiation followed by 2.31×10^9 nucleated marrow cells from her twin. For three days the patient had bloody diarrhea and nausea. Peripheral formed elements reappeared after 12 days. Platelets lagged behind leukocyte and reticulocyte recovery. The leukocyte count and differential were normal by the twenty-eighth day.

The dose of radiation used was twice the lethal dose for man. It is evident that successful transplantation of isologous marrow has been achieved. The harmful effects of radiation observed have been within tolerable limits. Success in these patients with isologous marrow and success in dogs with autologous marrow indicates that marrow recovery can be induced after doses of radiation capable of clinical usefulness in the control of disseminated neoplasia. Broad applicability of radiation and autologous marrow transplants to the clinical control of disease in man is anticipated.

Abnormal Permeability of Glomerular Capillaries in Hypertension and Diabetes. Louis Tobian,* John Cich and Odean Severseike, Minneapolis, Minn.

Hypertension and diabetes are often associated with proteinuria, hyaline glomerular deposits and hyaline in afferent arterioles, all conceivably caused by abnormal endothelial permeability. To assess the permeability of glomerular capillary endothelium, colloidal iron was injected into rats intravenously. Their kidneys were obtained four hours later and the iron deposits in the glomeruli were subsequently estimated by a semi-quantitative index. Sixteen normal Wistar rats had a mean iron index of 77 per 100 glomeruli. Fourteen other Wistar rats were made hypertensive by partially constricting one renal artery. These "ischemic" kidneys had a mean iron index of 24. The contralateral "untouched" kidney in these same rats had a mean iron index of 115. These indices were both significantly different from normal

($p < 0.01$), indicating that the kidney exposed to the hypertension had an endothelial permeability significantly greater than normal while the kidney protected from the hypertension by the clip had an endothelial permeability considerably less than normal. Permeability was definitely related to blood pressure. The increased permeability in kidneys exposed to hypertension could explain many of their functional and structural lesions.

Holtzman rats were made diabetic with alloxan. Three weeks later, the kidneys were obtained. The iron index of 18 normal Holtzman rats averaged 107. The iron index of 27 rats with moderate diabetes averaged 51, significantly lower than normal ($p < 0.00001$). The index in nine severely diabetic rats averaged 80, significantly higher than the moderate diabetics and lower than the normals. The data indicate a reduced permeability of the glomerular capillaries in rats with moderate alloxan diabetes, whereas rats with severe diabetes have a significantly higher capillary permeability, but still well below normal. This reduced permeability may explain why alloxan diabetic rabbits are unusually resistant to cholesterol-induced atherosclerosis and why alloxan diabetic rats resist intercapillary glomerulosclerosis.

Synthesis and Metabolism of Estrogens by Perfused Human Placentas. PHILIP TROEN, Boston, Mass. (Introduced by Herrman L. Blumgart).

Some of the unresolved problems in the endocrinology of pregnancy concern the factors controlling placental estrogen, the contribution of the placenta to estriol production, and the role of the placenta in producing the newly discovered estrogen metabolites. These problems have been approached by perfusion of the isolated human placenta using the technique previously described from this laboratory. Seven human placentas were perfused with C^{14}-estradiol or C^{14}-acetate, respectively, with and without added human chorionic gonadotropin (HCG). Estrogens were extracted from the perfusates and placental tissue and identified by paper chromatography, countercurrent distribution and derivative formation.

Following C^{14}-acetate perfusion, C^{14}-estradiol, C^{14}-estrone and C^{14}-estriol were found, in a yield of 0.01 per cent. Approximately equal amounts of estradiol and estrone were formed with a slightly greater yield of estriol. Increased synthesis of all three estrogens was suggested when HCG was added. This is the first demonstration of synthesis of estriol from acetate by human placental tissue. It is concluded that the major portion of this estriol does *not* arise as a metabolite of estradiol because here the ratio of estriol to estradiol is significantly higher than the ratio found when C^{14}-estradiol is converted to C^{14}-estriol as noted in our previous placental perfusion studies.

Following C^{14}-estradiol perfusion, C^{14}-2-methoxyestrone was produced, equivalent to about 3 per cent of the extracted C^{14}-estrone. There was also a 1 per cent conversion of C^{14}-estradiol to C^{14}-16-epiestriol. These two conversions were found only when HCG was added. This first demonstration of the ability of the placenta to produce these estrogen metabolites may explain the source of these metabolites in pregnancy urine.

These observations further indicate the role of HCG in placental function and self-regulation.

The Effect of X-Radiation upon Delayed Hypersensitivity and Antibody Formation in Guinea Pigs. JONATHAN W. UHR and MATTHEW SCHARFF, New York, N. Y. (Introduced by Lewis Thomas).

Whole body X-radiation given before antigenic stimulation is capable of eliminating the primary antibody response in various laboratory animals. The capacity of such radiated animals to develop the delayed type of hypersensitivity is not known. Guinea pigs of the Hartley strain were given 200 r. whole body X-radiation (approximately an LD 50) at various times before and after injection of protein antigens into the footpads. The surviving animals all showed weight loss and severe pancytopenia. Even when X-radiation was administered 24 hours *before* injection of 3 μg. diphtheria toxoid or ovalbumin in adjuvant (with or without killed mycobacteria) delayed-type skin reactions to 3 μg. of specific antigen were usually present seven days after sensitization. The delayed skin reactions were usually smaller than those seen in sensitized unirradiated control animals. The histology of the lesions, however, was typical of "tuberculin-type" skin reactions. Almost all of the sera obtained from toxoid-sensitive animals one week after the "boosting" skin test dose were incapable of eliciting passive cutaneous anaphylaxis or neutralizing toxin in the rabbit skin. Active cutaneous anaphylaxis performed on each animal did reveal antibody in a minority of the animals. Serum antibody appeared in many animals by three weeks coincident with other signs of recovery from the radiation effects.

These results indicate that the capacity to develop the delayed type of hypersensitivity in X-radiated guinea pigs may persist even when detectable circulating antibody is not produced two weeks after an immunizing injection of toxoid.

Magnesium Deficiency Tetany in Man. DAVID D. ULMER, WARREN E. C. WACKER and BERT L. VALLEE,* Boston, Mass.

Magnesium tetany in mammals is manifested by semicoma, severe neuromuscular hyperirritability including Chvostek's sign and carpo-pedal spasm, athetoid movements, marked susceptibility to auditory, mechanical and visual stimuli, a decreased magnesium and a normal serum calcium concentration. The syndrome, with only minor variations, has been clearly identified in a large number of animal species as a consequence of both spontaneous and experimental magnesium deficiency. The discrete constellation of signs, which can only be distinguished from hypocalcemic tetany by chemical means, is completely reversed by administration of adequate quantities of magnesium. The *magnesium tetany syndrome* repre-

sents one of the infrequent instances in which the consequences of a deficiency state occur in man in a form virtually identical to that detected in animals. Previous attempts to characterize this entity in humans have failed to distinguish tetany from the wide variety of neuromuscular aberrations which may be accompanied by hypomagnesemia. This confusion probably accounts for the nonuniformity of response to magnesium therapy reported by different observers.

We have now observed four patients with true magnesium tetany. In each instance the syndrome has occurred as a complication of either severe infection or surgery in malnourished patients, treated with magnesium-free parenteral fluids for prolonged periods. In each, the serum magnesium concentration was low and that of calcium was normal. Parenteral magnesium resulted in prompt and dramatic amelioration of all the manifestations of the *magnesium tetany syndrome*. Concomitantly, the serum magnesium concentration rose to normal values. Subsequently the syndrome alternately and predictably recurred or subsided with the institution or omission of magnesium administration.

Precise definition of the human *magnesium tetany syndrome* permits the clinical delineation of a group of patients for which magnesium therapy is specific and imperative.

Adrenal Cortical Function in the Newborn Period. Robert A. Ulstrom and E. Colle, Minneapolis, Minn. (Introduced by Lewis W. Wannamaker).

Plasma from the umbilical cord and from infants during the first week of life contains cortisol as the major circulating free adrenocorticosteroid. Cortisone is present in cord plasma, but not later. Cortisol levels, measured by specific isotope dilution, rise to a level twice that of cord plasma during the first 12 hours of life. The level then slowly decreases to about 5 μg. per 100 ml. until the end of the third day, when it rises. During the first week urinary cortisol levels are very low, as are levels of cortisol metabolites. The average daily value of total Porter-Silber chromogens increases each day during the first week. Of these, one-fourth to one-third are in the free form. The free fraction is composed mainly of steroids more polar than tetrahydrocortisol. 6 Beta hydroxycortisol has been identified as a major component of this fraction. Studies of this fraction after cortisol C^{14} administration indicate that 6 beta hydroxycortisol is a metabolite of cortisol.

Steroids rendered extractable after incubation with beta glucuronidase have not yet been shown to be glucuronide conjugated steroids. Omission of the active enzyme from the incubation mixture has not appreciably diminished yields of these in our studies. These "released steroids" have been studied qualitatively. None of our studies support the hypothesis that corticosterone is produced in relatively greater quantities in the neonate.

During the first week of life, there is little change in total urinary Porter-Silber chromogen excretion following stress, such as major surgical procedures. After the sixth day of life, however, such stressful procedures and the administration of corticotropin cause increased excretion such as is seen in the adult in comparable circumstances.

Demonstration of a Relationship between Insulin Binding and Insulin Action in the Peripheral Tissues of Normal Man. Roger H. Unger, Leonard L. Madison, Mary Sue McCall and Henry Lanz, Dallas, Texas. (Introduced by Ben Friedman).

The concept that insulin binding by certain tissues is prerequisite for its action therein was based upon demonstration of an *in vitro* correlation between insulin binding and action in the rat diaphragm.

The following experiments were designed to determine whether insulin binding by human peripheral tissues is demonstrable and, if so, whether it is related to insulin effect.

Twenty normal adults were given 0.5 unit of insulin-I^{131} by femoral artery and external radioactivity was recorded simultaneously over each calf. In all but one, marked inter-calf difference in radioactivity lasting longer than four hours was noted; inter-calf radioactivity ratio (injected side to opposite side) averaged 3.2 (range, 1.6 to 5.3). This difference reflects first-circulation intracellular insulin fixation in the injected extremity since: *1*) Injection of extracellularly distributed Na-I^{131} or $Na^{22}Cl$ uniformly gave more rapid inter-calf equalization with a mean inter-calf radioactivity ratio of 1.3 (1.1 to 1.5). *2*) Preinjection of unlabeled insulin into the artery reduced the mean radioactivity ratio following injection of insulin-I^{131} to 1.6 (1.8 to 2.2) and markedly shortened inter-calf equalization times, presumably by presaturating insulin binding sites on the injected side. *3*) In three insulin treated diabetics with intravascular retention of radioinsulin, indicated by markedly delayed plasma disappearance of the trichloracetic acid-precipitable radioactivity, low radioactivity ratios averaging 1.3 (1.0 to 1.7) were observed.

Insulin effect was determined during 11 of the above experiments by iodometric measurement of arteriovenous glucose difference in each leg. The "glucose uptake ratio" (injected leg to opposite leg) was calculated for each subject based on summated A-V glucose differences in each leg. The average ratio following insulin administration was 2.3 (0.4 to 4.5).

With one exception, greater insulin binding on the injected side was accompanied by greater insulin action on that side (glucose uptake ratio > 1.0), thus providing the first indication in man of a correlation between insulin binding and insulin action.

The Role of the Left Atrial Stretch Receptor Mechanism in Carbon Dioxide Diuresis. H. Valtin, I. D. Wilson and S. M. Tenney,* Hanover, N. H.

Carbon dioxide diuresis probably is mediated via a nonosmotic influence on the supraopticohypophysial system, resulting in decreased secretion of antidiuretic hor-

mone. Since the left atrial stretch receptor mechanism is one such nonosmotic system which CO_2 might influence, experiments were designed to elucidate the role of this mechanism in CO_2 diuresis.

In man breathing 6 per cent CO_2 the diuresis is abolished by blood pooling in the lower extremities, for instance, in the erect posture or in the supine posture with venous occlusive tourniquets high on both thighs. Prevention of blood pooling in the erect posture, as on mild exercise or on standing in water to the level of the symphysis pubis, restores the diuretic effect.

Voluntary hyperventilation mimicking that which accompanies CO_2 inhalation results in a much smaller diuresis and one which, unlike that of CO_2, is accompanied by increased sodium excretion. Maintaining normal alveolar CO_2 tension during voluntary hyperventilation by simultaneous inhalation of 2 per cent CO_2 in no wise alters this result.

Cats breathing 12 per cent CO_2 show typical diuresis in the absence of significant change in plasma or total blood volume. Section of both vagus nerves, in which afferent impulses from the left atrial stretch receptors are carried, does not abolish CO_2 diuresis, but may enhance it.

These data strongly suggest that the left atrial stretch receptor mechanism is not the afferent system for CO_2 diuresis. The diuresis probably is prevented by extremity blood pooling because the smaller left atrial volume inhibits the CO_2 diuretic effect.

The Effect of High Carbohydrate Intake and of Hydrochloric Acid Administration on Fat Absorption in Gastrectomized Patients. PARKER VANAMEE, WALTER LAWRENCE, JR., SAM LEVIN, ANN S. PETERSON and HENRY T. RANDALL, New York, N. Y. (Introduced by Rulon W. Rawson).

It is commonly recognized that there may be a defect in fat absorption following total or subtotal gastrectomy. The etiology of the steatorrhea has been variously attributed to loss of the reservoir function of the stomach, loss of the digestive and triturative functions of the stomach, decreased stimulation of the pancreatic and biliary secretions, inadequate mixing of food with the pancreatic and biliary secretions, and increased intestinal motility. Previous studies in this laboratory have indicated that the percentile loss of fat in the stools remains constant over a wide range of fat intakes. The present study indicates that the defect in fat absorption may be increased by a high carbohydrate diet. Hydrochloric acid administration caused an improvement in fat absorption in the majority of the patients, suggesting that the absence of the acid contributes either directly or indirectly to the steatorrhea.

Twenty-seven patients who had been subjected to subtotal or total gastrectomy were placed on metabolic balance study. During the control periods 13 of these patients showed a defect in fat absorption. Thirteen patients, including six patients with normal fat absorption in the control period, showed a decrease in fat absorption of from 4 to 54 per cent when challenged with a high carbohydrate diet.

Thirteen patients demonstrating mild to moderate defects in fat absorption while on a high carbohydrate diet were given 25 ml. of 0.1 normal hydrochloric acid five minutes prior to meals for a three day metabolic block study. There was an increase in fat absorption in nine of the 13 patients when compared to a preceding period on the same diet. The improvement in absorption ranged from 7 to 13 per cent. Five of these patients had improvement in fat absorption sufficient to bring them into normal range.

The Thyroid Suppression Test in the Prognosis of Hyperthyroidism Treated with Antithyroid Drugs. W. P. VANDERLAAN * and CARL CASSIDY, La Jolla, Cal. and Boston, Mass.

Failure of the administration of thyroid U.S.P. to suppress radioiodine uptake by the thyroid gland partially characterizes hyperthyroidism. Previously we observed normal suppression tests in 10 of 12 patients six to 13 years after successful treatment of hyperthyroidism with antithyroid drugs.

The present study related the thyroid suppression test to the prognosis of hyperthyroidism treated with antithyroid drugs. The patients were treated for one year, usually with propylthiouracil. In the last three weeks of treatment thyroid U.S.P., 0.2 Gm. per day, was also given. Radioiodine uptake per 24 hours was then measured, and all patients were observed.

Thirty-two hyperthyroid patients undertook 33 courses of treatment. Five patients experienced six relapses in four months or less (average, six weeks). Twenty-seven patients had remissions lasting six months or more (average, 14 months, to date). The original, diagnostic uptake values in the relapsing group (70 per cent $\pm$ 10) and in the group with remissions (71 per cent $\pm$ 18) were similar. After treatment and attempted thyroid suppression radioiodine uptake values averaged 80 per cent ($\pm$ 17) in those who relapsed, in contrast to 31 per cent ($\pm$ 18) in those who remained well. Among the 11 instances where radioiodine uptake exceeded 50 per cent six relapses into hyperthyroidism occurred.

The thyroid suppression test at the end of one year of treatment with antithyroid drugs gave a highly significant difference between the group which sustained a lasting remission and that which relapsed promptly. Application of these findings should prove helpful in selecting the time to conclude antithyroid therapy.

The Effect of Epinephrine, Glucagon and Adrenocorticotropic Hormone (ACTH) on Phosphorylase Activity in Adipose Tissue. MARTHA VAUGHAN, DANIEL STEINBERG and ELEAZAR SHAFRIR, Bethesda, Md. (Introduced by Donald S. Fredrickson).

The mechanism by which these hormones stimulate release of unesterified fatty acids from adipose tissue is unknown. Epinephrine and glucagon in liver and ACTH

in adrenal increase the accumulation of 3′, 5′ adenosine monophosphoric acid. This accelerates the conversion of inactive to active phosphorylase. Studies were undertaken to determine whether a similar action was demonstrable in adipose tissue.

Phosphorylase activity was determined by the method of Sutherland in homogenates of rat epididymal fat bodies. Glycogen-iodine color increased concomitant with inorganic phosphate formation. Suitable controls also indicated that phosphate formed in the assay resulted from phosphorylase action. No glucose-6 phosphatase activity was detected. Phosphorylase activity of adipose tissue incubated at 37° declined in 30 minutes to 50 per cent of the initial value. Epinephrine inhibited this decrease. Hormone effects, the difference between enzyme activities of paired tissues from a single rat incubated simultaneously one with and one without hormone, expressed in μg. P per 100 mg. tissue per 30 minutes (mean ± standard error) were: epinephrine (0.8 μg. per ml.), + 38 ± 7.7; glucagon (50 μg. per ml.), + 29 ± 10.4; ACTH (20 μg. per ml.), + 42 ± 12.2. All of these hormone effects are significant ($p = < 0.025$). Glucagon free insulin (8 μg. per ml.) was without effect. An effect of 3′, 5′ adenosine monophosphoric acid suggested that in adipose tissue also it may mediate the action of these hormones. In liver, epinephrine and glucagon cause glycogenolysis and glucose release. In adrenal, it is postulated that ACTH induced glycogenolysis leads to increased corticosteroid synthesis. It is tempting to consider that in adipose tissue also phosphorylase is a component of the mechanism by which the three hormones enhance the output of unesterified fatty acids.

Effects of Acute Hypercalcemia in Dogs. STANLEY WALLACH and ANNE C. CARTER, Brooklyn, N. Y. (Introduced by David M. Kydd).

Hypercalcemia produces metabolic effects other than to inhibit parathormone secretion and increase tubular reabsorption of phosphate. In the present study the effects of elevated serum calcium levels on glomerular filtration rate, renal plasma flow, osmolal and free water clearance, plasma and urine concentrations of sodium, potassium, calcium, magnesium, ammonia, titratable acid, pH and inorganic phosphate were made in dogs. Red blood cell calcium, magnesium and acid soluble phosphate concentrations were determined. Control measurements were made during saline infusions, followed in two to four weeks by the intravenous administration of calcium gluconate in saline.

Hyperphosphatemia, unaccompanied by alterations in renal function or increased tubular reabsorption of phosphate, appeared immediately after the induction of hypercalcemia. Marked augmentation of sodium, potassium, magnesium, titratable acid and ammonia excretion occurred without increased plasma concentration or glomerular filtration of these ions. Free water clearance was not changed appreciably. Red blood cell calcium increased one- to threefold, with no significant change in intracellular magnesium or acid soluble phosphate. Elevations of plasma calcium concentrations above 18 mg. per 100 ml. resulted in impaired renal plasma flow, glomerular filtration rate and renal concentrating ability, all of which were reversible. Similar responses have been observed in normocalcemic vitamin D treated and euthyroid hypocalcemic dogs.

The hyperphosphatemia and generalized cation diuresis cannot be explained solely by the effect of hypercalcemia on renal tubular function. It would appear that during induced hypercalcemia, calcium enters cells and the hydration layer of bone crystals resulting in heterionic exchange and transfer of other cations and phosphate from these sites to the extracellular space. Associated changes in renal tubular function permit rapid excretion of the transferred cations. An analogous process involving intracellular heterionic exchange and alteration in renal tubular function is believed to occur during acid loading.

The Ionic Composition of Uremic Plasma. MACKENZIE WALSER, Baltimore Md. (Introduced by Gilbert H. Mudge).

Although it is known that plasma magnesium is increased and plasma calcium decreased in renal failure, the interaction of these ions with protein, phosphate and citrate hinders estimation of their effective concentrations.

The method of Raaflaub for determining free calcium ions, using purpurate (murexide), was reinvestigated and found to be accurate and specific. Neither Na, K, Mg, Cl, HCO_3, heavy metals nor the dye itself introduce significant errors. Reciprocal absorbance is a linear function of reciprocal calcium ion concentration. The parameters of this function change predictably with pH and ionic strength.

Eriochrome black T was found to fulfill the same criteria as a measure of free magnesium ions, being unaffected by calcium ions under physiological conditions. Simple multivalent anions were found to reduce free calcium and magnesium ion concentrations by electrostatic association.

Ultrafiltrates of normal and uremic plasma samples were analyzed by these methods. Determination of phosphate and citrate, as well as pH, permitted calculation of the portions of these anions which were free or which were complexed with Ca and Mg.

The following statistically significant differences were observed between uremics and normals: decreased calcium ions (1.04 *vs.* 1.42 mM per L.); increased magnesium ions (0.61 *vs.* 0.35 mM per L.); increased HPO_4 ions (2.04 *vs.* 0.80 mM per L.); decreased citrate ions (0.025 *vs.* 0.032 mM per L.). The fraction of ultrafiltrable magnesium present as free ions decreased (57 *vs.* 73 per cent), as did that of calcium (86 *vs.* 96 per cent). The concentration of the complex $MgHPO_4$ was increased fourfold, and that of $CaHPO_4$ twofold. In the most severe uremics, undetermined magnesium complexes also appeared in considerable amounts. These observations may be relevant to the pathogenesis and management of the uremic syndrome.

Tracer Studies of Plasma Lipids in Human Subjects. CHRISTINE WATERHOUSE* and GUIDO MARINETTI, Rochester, N. Y.

Plasma lipids of normal individuals and of patients with the nephrotic syndrome were investigated by column and paper chromatography employing silicic acid as the stationary phase. Triolein I^{131} was given in tracer amounts to six of the subjects. Radioactivity was found in the column fractions containing neutral fat, cholesterol ester and unesterified fatty acids and in smaller amounts in the fractions containing phosphatidylethanolamine, lecithin, sphingomyelin and lysolecithin; however, the total activity in these known lipid components accounted for only a relatively small percentage of the total activity of the lipid extracts. This discrepancy became more marked as the time interval increased after the administration of the label (up to 72 hours).

A highly active component was separated from all other plasma lipids by combination of column and paper chromatography and finally by differential solubility. Although the active component was extracted by common lipid solvents, when isolated as crystalline material it was only slightly soluble in chloroform but soluble in methanol or water. While elution of this fraction from the column occurred with the phospholipids, this compound is free from phosphorus but is high in nitrogen. Nearly identical infrared spectra of the material were obtained on samples from three individuals. The major absorption bands are characteristic for C=O and NH groups. Acid hydrolysis yielded an ether-extractable, nitrogen-free substance which chromatographically had the properties of a lipid. The aqueous phase contained components high in nitrogen, but with only trace amounts of ninhydrin-positive compounds. Starch-block electrophoresis of whole plasma containing 90 per cent of the total radioactivity in this unknown lipid failed to reveal radioactivity in any of the proteins indicating the highly active component is not firmly bound to protein.

At present we have no conclusive evidence as to the nature of the lipid material in the newly isolated fraction. However, its high activity implies biological importance in lipid transport and its solubility properties may indicate an important role in cellular penetration of fatty acid materials.

Carbonic Anhydrase Inhibition in Renal Tubular Acidosis. G. D. WEBSTER, JR. and E. J. HUTH, Philadelphia, Pa. (Introduced by J. R. Elkinton).

The renal response during the second and third hour after a single dose (10 mg. per Kg.) of acetazolamide (Kaye's test) was compared quantitatively in the following groups: *a*) 10 normal subjects, *b*) three patients with renal tubular acidosis (R.T.A.) on alkali therapy, *c*) four non-acidotic patients with generalized renal disease (chronic glomerulonephritis, nephrosclerosis), and *d*) four chronically ill patients with no evidence of renal disease except moderate depression of endogenous creatinine clearance.

The urinary pH rose in all groups. The mean and ranges of $\Delta UV_{HCO_3^-}$ in μEq. per minute per 1.73 $M.^2$ for the four groups were as follows: *a*) +306 (+205 to +548), *b*) +100 (+36 to +139), *c*) +89 (+32 to +126), and *d*) +140 (+78 to +206). The corresponding values for ΔUV_H+ ($^-UV_{TA+NH_4^+-HCO_3^-}$) were within a few μEq. of the above values, with a change of sign; the values for both moieties in Groups *b* and *c* were entirely below the ranges of the corresponding values in Group *a*. Changes in HCO_3^- reabsorption in mEq. per L. filtrate were as follows: *a*) −5.6 (−3.9 to −9.2), *b*) −2.1 (−0.9 to −2.5), *c*) −1.9 (+0.3 to −4.0), *d*) −4.8 (−3.1 to −5.8). Absolute values for $\Delta HCO_3^-{}_{reab.}$, and hence for change in total H^+ secretion (assuming all HCO_3^- reabsorption involves an equivalent H^+ transfer), indicated the same relationship between the two groups, *i.e.*, a lesser response to the enzyme inhibitor in Groups *b* and *c* as compared to *a* and *d*.

Despite a positive correlation between HCO_3^- filtered and the changes in HCO_3^- reabsorbed and excreted, the above data suggest: *1*) that under standardized conditions of enzyme inhibition, renal carbonic anhydrase activity was the rate limiting factor in HCO_3^- reabsorption, H^+ secretion and H^+ excretion, and *2*) that the activity of this enzyme per unit filtrate was diminished in both the R.T.A. patients and the patients with generalized renal disease.

Changes in Acid-Base Balance and Plasma Electrolytes after Administration of Sodium Cycle Resin. JOHN M. WELLER, Ann Arbor, Mich. (Introduced by Sibley W. Hoobler).

Ingestion of 20 Gm. sodium cycle carboxylic acid resin by six subjects elevated the plasma pH into the alkalotic range. This alkalosis was in part metabolic (an increase in buffer base concentration), but at eight and 24 hours after administration of resin it was largely respiratory (a decrease in partial pressure of carbon dioxide). The greatest per cent change in plasma electrolyte was that of potassium which decreased 0.66 mEq. per L. eight hours after ingestion of resin.

Similar elevation of the pH occurred in two subjects who took 30 Gm. sodium bicarbonate. This alkalosis was also primarily metabolic in type, but one, in addition, had a respiratory alkalosis. Their plasma potassium concentration also decreased.

Lowering of the potassium results from the alkalosis due to the resin removing hydrogen ions from the upper gastrointestinal secretions, as well as by its removing potassium from the body. It is difficult to separate the direct removal from the indirect effect due to elevation of the pH. In comparing the changes in plasma potassium after resin with those after sodium bicarbonate, it is apparent in four of the six subjects given resin that the magnitude of change in potassium in relation to the change in pH was greater than the similar change in individuals taking sodium bicarbonate. In these four subjects removal of potassium by the resin was a significant factor superimposed on the shift of potassium from extracellular into cellular fluid secondary to alkalosis.

Effects of Cold Air Upon the Normal and Abnormal Respiratory Tract. ROE E. WELLS, JR., JAMES E. C.

WALKER and ROGER B. HICKLER, Boston, Mass. (Introduced by Eugene C. Eppinger).

Numerous studies have testified to the ability of normal man to operate under extreme arctic conditions without loss of functional reserve or injury to his respiratory system. However, scant attention has been paid to the causes of the incapacitating symptoms that develop in patients with chronic pulmonary disease when exposed to the cold. A study of this phenomenon would require a separation of the stimulus of cold air inhalation from that of cold air exposure to the body surface. To analyze the effects of these stimuli separately and in combination techniques were devised that permitted continuous recording of the airway temperature and the mechanical events of respiration while the subject breathed cold or warm air in a cold or warm environment. Measurements of pulmonary compliance, airflow resistance and the work of breathing were carried out by the intraesophageal balloon technique. Respired air temperatures (range, -40 to $+32°$ C.) were monitored by thermocouples of rapid response time.

Studies of 10 normal subjects revealed no significant change in respiratory function during cold inhalation or exposure. No symptoms were noted in these subjects when breathing air of $-40°$ C. Studies of 20 subjects with chronic pulmonary disease, primarily asthma, bronchitis and emphysema, demonstrated dramatic increase in respiratory airflow resistance and work of breathing. These changes were frequently associated with distressing symptoms of cough, wheezing, rhinorrhea and salivation. Cold air exposure during breathing of warm room air (25° C.) did not result in significant changes in airflow resistance whereas the increased resistance that developed upon the inspiration of cold air was frequently greater when cold air exposure was added to this. A nebulized bronchodilator (Isuprel®) was effective in reducing the elevated airflow resistance as well as preventing bronchospasm in subsequent cold air inhalations.

Lipid Quantification in Normal Young and Normal Aged Circulating Erythrocytes. MAXWELL P. WESTERMAN, LAWRENCE E. PIERCE and WALLACE N. JENSEN,* Pittsburgh, Pa.

A consideration of the contribution of lipids to erythrocyte integrity necessitates identification and quantification of these substances in circulating cells of various ages. These results, for meaningful interpretation, must be related to an appropriate cell parameter. To evaluate these concepts, separation and quantification of lipids in young and old cell populations were accomplished and results expressed as concentration per cell and per unit of surface area.

Ten normal individuals were studied. Duplicate determinations of erythrocyte cholesterol, phospholipid and individual phosphatides were made. Erythrocyte population separations were achieved by centrifugation and verified by the relative centrifugation position of reticulocytes and labeled cells of known age. Lipid partitions were accomplished by chromatography and individual specific phosphatide quantities determined after elution. Recovery ranged between 97 and 106 per cent.

Surface area (μ^2) was significantly larger ($p = 0.01$) in younger (150 ± 5.2) than in older (140 ± 8.5) cells. Total lipid per young cell ($479 \pm 85 \times 10^{-12}$ mg.) was greater ($p = 0.05$) than in old cells (409 ± 54). Total phospholipid concentration was $300 \pm 45 \times 10^{-12}$ mg. in young cells and 256 ± 32 per old cell ($p = 0.02$). Cholesterol content was higher in young ($112 \pm 6 \times 10^{-12}$ mg.) than old (94 ± 7 cells ($p = < 0.001$)). When total lipid and phospholipid are related to surface area, the differences between young and old cells are not significant in contrast to the persistence of significant differences ($p = 0.02$) in cholesterol content. Individual phosphatidyl ester percentages of the total phospholipid were similar in both populations.

The significant loss of erythrocyte lipid with aging is evidently not related to the concentration of surface or membrane lipid except for cholesterol which shows significant differences with reference to either the individual cell or surface area. Whether or not significant "functional lipid" is lost cannot be concluded from these data. These observations provide a basis for comparison with abnormal cells and a background for kinetic studies.

The Effects of 3:5:3′ Triiodothyropropionic Acid on Serum Lipids of Hypercholesterolemic Euthyroid and Hypothyroid Subjects. RAYMOND E. WESTON,* Beverly Hills, Cal.

The well-known relationship between thyroid activity and lipid metabolism has stimulated interest in synthetic thyroid analogues which may be less calorigenic but still reduce serum lipid concentrations. In the present study, three hypothyroid, one panhypopituitaroid and 12 euthyroid subjects were given 3:5:3′ triiodothyropropionic acid (triprop), reported to maintain myxedematous subjects euthyroid in three to six mg. per day doses. Two hypothyroid and three euthyroid patients had coronary artery disease. All were hypercholesterolemics, whose serum lipids had decreased somewhat on weight reduction, low fat intakes, and, in three cases, sitosterol therapy. Placebos were given until serum cholesterol levels stabilized. Observations of body weight, serum cholesterol and protein bound iodine concentrations were made at weekly and, later, biweekly intervals. Electrocardiograms, basal metabolic rates and serum lipid spectra were taken periodically.

Hypothyroid patients exhibited serum cholesterol decreases on triprop doses (1 or 2 mg. per day), without effect on body weight, pulse rate or BMR. Thus, serum cholesterol of one such patient with severe coronary insufficiency fell from 453 to 200 mg. per cent in 10 days, when 1 mg. of triprop was given in addition to 0.2 mg. L-thyroxine daily maintenance dose, and remained between 250 and 260 mg. per cent on 2 mg. of triprop per day after L-thyroxine was discontinued. In the

other three hypothyroids, BMR increased and weight decreased progressively on 3 to 6 mg.

Euthyroid subjects also exhibited reductions in serum cholesterol levels on triprop (1 to 12 mg. per day) without increase in BMR or weight loss. Thus, the average serum cholesterol of the 16 patients fell from 295 to 222 mg. per cent (22.6 per cent). One euthyroid patient exhibited nervousness and tremor on 6 mg. per day. Another developed generalized urticaria, which promptly subsided on discontinuation of triprop. No increased cardiac symptoms were noted. More extended studies on the effects of triprop in hypercholesterolemic patients with coronary disease are in progress.

Determinants of Bile Secretion in the Dog. HENRY O. WHEELER and OSWALDO RAMOS, New York, N. Y. (Introduced by Franklin M. Hanger).

The flow and composition of bile are profoundly affected by variations in bile acid secretory rate. When the latter is kept constant additional determinants can be evaluated. During constant intravenous sodium taurocholate infusion (*circa* 13 μmMole per minute) bile was collected anaerobically from four fasting unanesthetized cholecystectomized dogs by Thomas fistula. Electrolytes, bile acids, pH and freezing point were measured. Bile acid concentration $[A^-]$ proved equal to the anion-cation deficit: $[A^-]/[Na^+ + K^+) - (Cl^- + HCO_3^-)] = 0.97 \pm$ S.D. 0.09. The sum $[Na^+] + [K^+] + [Cl^-] + [HCO_3^-]$ (average, 295 mEq. per L.) and the freezing point were constant and independent of $[A^-]$. Thus bile acids do not appear to contribute to osmolarity in proportion to their molecular concentration.

Despite constant bile acid excretory rate, bile flow still exhibited marked spontaneous variations. Increments were accompanied by increases in $[HCO_3^-]$ (range, 6 to 60 mEq. per L.), pH (6.7 to 7.8) and $[Cl^-]$ (27 to 102). In each animal composition at a given flow was constant from day to day. Highest flows, pH and $[HCO_3^-]$ were achieved with intravenous secretin (*circa* 5 U per Kg. B.W.) or intraduodenal HCl administration. Negligible amylase content ruled out contamination by pancreatic juice.

Thus bile could be regarded as a mixture of two isosmotic fractions: *1*) bile acid and other constituents secreted by hepatic parenchymal cells with equilibration of water presumably by passive diffusion, and *2*) a solution whose electrolyte composition and secretin response resemble that of pancreatic juice. Electrolyte concentrations in Solution *2* calculated on this assumption change with increments in flow as follows: $[HCO_3^-]$ rises (13 to 75 mEq. per L.); $[Cl^-]$ falls (129 to 77). Variations in bile flow and composition could be attributed to the variable output and composition of the second fraction when bile acid excretion is constant.

On the Mechanism of Toxicity of Nitrogen Mustard. LAURENS P. WHITE, San Francisco, Cal. (Introduced by Lowell A. Rantz).

This report presents evidence that the lethal effects of nitrogen mustard are indirect, and are mediated by a toxic product derived from the interaction of mustard and certain proteins.

Previous studies in this laboratory have demonstrated that prior splenectomy can prevent death in dogs given a dose of nitrogen mustard which is lethal in control animals. In mice the intraperitoneal injection of an incubated mixture of splenic homogenate and nitrogen mustard has proven uniformly lethal, while injection of the same amount of mustard without splenic homogenate is lethal in only 10 per cent of animals, and homogenate by itself is nontoxic.

Attempts to identify the toxic material form the basis of this report. Homogenates of various tissues in saline were incubated with nitrogen mustard (HN2) for 15 minutes at room temperature. Several proteins and amino acids were also incubated with HN2. In one study ultracentrifugal separation of sucrose homogenates of spleen was performed, and the individual fractions incubated with nitrogen mustard. For each study equivalent amounts of nitrogen mustard by itself, and of the test substance alone, were injected into control animals. In summary our results demonstrate that: *1*) The toxic material is found in mustard treated homogenates of spleen, liver, kidney, muscle and blood, but in greatest amount in splenic homogenates. *2*) The material is heat stable and is not retained by dialysis in cellophane, although undialyzed material of the same age retains toxicity. *3*) The material is found in every cellular fraction (nuclear, mitochondrial, microsomal and soluble) but in greatest amount in the soluble fraction. *4*) The material is formed rapidly upon incubation of nitrogen mustard with a variety of proteins, notably human serum albumin, but not with several amino acids.

The Mechanism of Venous Pressure Elevation with Exercise in Human Congestive Heart Failure. J. EDWIN WOOD, Augusta, Ga. (Introduced by Thomas Findley).

The roles of the peripheral veins and the autonomic nervous system in producing venous pressure rise with exercise in cardiac failure were investigated.

Twelve recumbent decompensated patients were studied before and after intravenous pentapyrrolidinium bitartrate (2 to 6 mg.). Preceding autonomic blockade mean venous volume per 100 cc. forearm per 30 mm. Hg rise in local effective venous pressure measured plethysmographically before exercise was 2.56 cc. (S.D. 0.58) and was 1.28 cc. (S.D. 0.42) during exercise with a pedal device ($p < 0.01$). Mean venous pressures referred to the right atrium were 15.5 mm. Hg (S.D. 6.2) and 24.0 mm. Hg (S.D. 4.9), respectively ($p < 0.01$). Following pentapyrrolidinium mean venous volume before exercise was 2.82 cc. (S.D. 0.61) and 2.79 cc. (S.D. 0.64) during exercise ($p > 0.5$). Mean venous pressures were 13.1 mm. Hg (S.D. 5.5) and 14.6 mm. Hg (S.D. 5.4), respectively ($p > 0.5$).

These studies were repeated in six of these patients during circulatory arrest of the exercising limbs. Mean

venous volume before exercise was 2.90 cc. (S.D. 0.58) and 1.60 cc. (S.D. 0.49) during exercise ($p < 0.01$). Mean venous pressures were 14.5 mm. Hg (S.D. 6.0) and 22.3 mm. Hg (S.D. 4.9), respectively ($p < 0.02$). Following pentapyrrolidinium mean venous volume before exercise was 2.88 cc. (S.D. 0.26) and 3.00 cc. (S.D. 0.47) during exercise ($p > 0.5$). Mean venous pressures were 13.3 mm. Hg (S.D. 5.4) and 14.1 mm. Hg (S.D. 6.2), respectively ($p > 0.5$). These six patients rebreathed 2 L. of oxygen for 60 seconds. This also caused venoconstriction with venous pressure increase which could be blocked with pentapyrrolidinium. Comparable hyperventilation without rebreathing did not produce this response.

None of the above responses to exercise, ischemic exercise or rebreathing occurred in nine compensated patients who had been decompensated.

These results indicate that exercise causes venoconstriction and venous pressure rise during heart failure. The stimulus for venoconstriction is mediated via the autonomic nervous system and initiated by an afferent impulse from the exercised limb in response to the effects of local ischemia (possibly lowered pH) rather than local hemodynamic changes. This neuroeffector system is more sensitive in decompensated patients than in compensated patients.

Susceptibility to Experimental Pyelonephritis During and After Potassium Depletion. JAMES W. WOODS, LOUIS G. WELT,* WALTER HOLLANDER, JR.* and MARGARET NEWTON, Chapel Hill, N. C.

It is established that potassium depletion produces renal lesions in experimental animals and in man. A *temporary* deficiency of potassium has been shown to lead to permanent renal change in the rat despite subsequent repletion. Clinical data suggest an association between potassium nephropathy and pyelonephritis but this remains only suggestive.

Sixty-eight young, male Sprague-Dawley strain rats were divided into four groups, A(10 rats), B(20), C(10) and D(28). Each group was divided into an experimental and a control subgroup. The experimental animals in each group were placed on a potassium-deficient regimen for four weeks. The animals of Group A were then sacrificed and analyses of sera and skeletal muscle confirmed severe potassium depletion. The animals of Group B were given an intravenous inoculum of *E. coli*. Three K-depleted rats died within 18 hours. The survivors were sacrificed in eight or nine days. There was cultural or microscopic evidence of renal infection in six of seven experimentals but in none of 10 controls.

After potassium depletion, Groups C and D were placed on diets adequate in potassium. After six months the animals of Group C were sacrificed for analyses of the electrolyte content of sera and skeletal muscle and for histologic study. After seven months, the animals of Group D were inoculated with *E. coli* and sacrificed in eight or nine days. In the latter group, seven of 14 experimentals had gross kidney abscesses; 10 were positive by culture, and nine had histologic evidences of pyelonephritis. In contrast, only two of 14 controls had positive cultures and a third had microscopic evidence of pyelonephritis.

These data demonstrate that the nephropathy of acute K-depletion and the residual lesions following repletion render the rat kidney more susceptible to experimental pyelonephritis.

The Quantitative Measurement of Joint Stiffness. VERNA WRIGHT and RICHARD J. JOHNS, Baltimore, Md. (Introduced by A. McGehee Harvey).

A major manifestation of many forms of arthritis is joint stiffness. In rheumatoid arthritis subjective stiffness is characteristically most apparent in the morning. One of us (V. W.) had previously investigated the diurnal variation in muscle strength in the hand and demonstrated morning weakness. Thus, it was possible that joint stiffness was constant throughout the day and that the subjective "morning stiffness" was in fact morning weakness. Normal subjects, however, exhibited the same diurnal strength pattern. In order to evaluate this problem it was necessary to develop a method for quantitative measurement of joint stiffness in precise physical terms.

Joint stiffness was studied using a device which imposed a passive sinusoidal rotation upon the metacarpophalangeal joint. Both the amplitude and the frequency of this rotation were varied. The torque required to impose this motion provided a quantitative measure of the joint forces resisting motion. Torque was recorded and was related to the rotary displacement, velocity and acceleration of the joint. From these data was derived the joint stiffness attributable to elasticity, plasticity, friction, viscosity and inertia.

Comparison has been made between these parameters in normal subjects and in patients with various connective tissue diseases. The joint was found to behave as a visco-elastic solid exhibiting characteristic nonlinear elasticity, creep and stress relaxation. The nonlinear elasticity was the major factor in stiffness. Elastic stiffness was found to increase with age, cold, ischemia and rheumatoid arthritis; it was decreased with heat, with steroid treatment of rheumatoid arthritis, and in such conditions as the Ehlers-Danlos syndrome and osteogenesis imperfecta.

Partial Cardiopulmonary By-Pass Utilizing Selective Veno-Arterial Perfusion. MILFORD G. WYMAN, MAX H. WEIL and DAVID H. BLANKENHORN, Los Angeles, Cal. (Introduced by A. F. Rasmussen, Jr.).

Recent studies have indicated that the work load on the heart may be reduced by partial cardiac by-pass. Present experiments in anesthetized dogs were designed to test the feasibility of reducing cardiac work by veno-arterial perfusion without oxygenation. Fifteen perfusion experiments were performed and detailed physiological measurements were completed in six animals. A catheter

was advanced from each femoral vein into the inferior vena cava. Venous blood was passively drained into a reservoir maintained below the level of the heart. Blood was returned from the reservoir utilizing a Sigmamotor® pump and infused through a femoral artery catheter into the abdominal aorta near its bifurcation. The system was adjusted to deliver approximately one-half of the total cardiac output.

Dye dilution and oxygen saturation measurements indicated that there was effective division of upper and lower body circulations in the normotensive animal with abrupt change in oxygen saturation occurring approximately 15 cm. cephalad to the tip of the arterial catheter. A prominent continuous murmur was detected in this area. There was a 45 per cent reduction in cardiac output (dye dilution technique). The sum of the cardiac output and perfusion flow approximated the control cardiac output value. Pulse rate was reduced 25 per cent. Mean arterial pressure decreased from an average value of 135 to 115 mm. Hg. during the perfusion period. Venous pressure remained unchanged. Respiratory rate increased 35 per cent. Blood pH was unchanged or slightly lowered and no constant change in the carbon dioxide content was noted. A gradual re-establishment of normal cardiac output, arterial pressure, heart rate and respiration occurred during the hour following perfusion. Perfusion periods in excess of three hours were compatible with survival.

These studies suggest that selective veno-arterial perfusion without oxygenation provides a simple and accessible method of reducing cardiac output for a limited period of time.

Characterization of the Serum I^{127} Iodoaminoacids. JAMES WYNN, Durham N. C. (Introduced by R. Wayne Rundles).

The circulating I^{127} compounds in human serum have been characterized by their differential solubilities in alcohol and water. Accordingly, Treverrow (J. biol. Chem. 1938, **176**, 639) described "thyroxine-like" and "diiodotyrosine-like" fractions in the serum. Her work has been confirmed. In general, I^{131} studies have demonstrated thyroxine and 3:5:3′ triiodothyronine but no diiodotyrosine in human serum. Chromatographic methods have not been heretofore available for the analysis of I^{127} constituents of the serum.

A method of extraction, concentration and column chromatography of small amounts of labeled iodoaminoacids using an anion exchange resin has been described from this laboratory (V.A. Annual Research Meeting, 1958). This method has been modified to permit direct determination of organically bound iodine^{127} in the serum.

Results of studies of the iodinated compounds in normal serum are presented. Fifty ml. of serum was extracted and the iodoaminoacids fractionated by resin column chromatography. Fractions eluted from the resin were further characterized by ascending paper chromatography in three solvent systems. Iodide^{127} quantitation was carried out on the serum and on fractions from resin.

I^{127} thyroxine comprises 80 to 90 per cent of the PBI^{127} of normal serum. Ten to 20 per cent of PBI^{127} is composed of at least two components which have been chromatographically separated but not identified. These compounds do not appear to be iodotyrosines, 3:5 diiodothyronine, 3:5:3′ triiodothyronine or thyroxine. We find no circulating I^{127} iodotyrosines in normal serum. The sum of iodide recovery from all fractions represents 95 to 115 per cent of the PBI^{127} of the serum.

Hyperpolarization of Muscle Induced by Insulin in the Absence of Glucose. KENNETH L. ZIERLER,* Baltimore, Md.

The electrical potential difference (E_r) across the membrane of resting skeletal muscle is determined in part by the ratio of activity of potassium inside the muscle fiber $(K^+)_i$ to its activity outside the fiber $(K^+)_o$. By the same token, a change in E_r may be expected to produce a corresponding change in the ratio $(K^+)_i/(K^+)_o$. When rat muscle is maintained in saline-glucose solutions so that $(K^+)_o$ is held constant, insulin increases E_r. This insulin-induced hyperpolarization appears to precede increase in $(K^+)_i$, suggesting that hyperpolarization is the cause of potassium movement. Because there was the possibility that potassium movement in response to insulin may have been secondary to an effect of insulin on glucose movement or metabolism, experiments were repeated with glucose omitted from the bathing solution, but with cytidine present. Insulin caused about the same degree of hyperpolarization in the absence of glucose as in its presence. Changes in $(K^+)_i$ were inadequate to account for the observed increase in E_r. It is concluded that insulin-induced hyperpolarization is independent of insulin's effect on glucose uptake and that insulin-induced movement of potassium into muscle is independent of glucose uptake and secondary to increased E_r.

Unusual Chromatographic Behavior of Serum Macroglobulins. MORRIS ZIFF* and JOSEPH LOSPALLUTO, Dallas, Texas.

It has been possible to separate the gamma globulins of differing molecular size in normal and pathological sera by chromatography on a cellulose anion-exchanger (DEAE cellulose). In general, gamma globulin with a sedimentation coefficient of 7S (molecular weight, 150,000), which constitutes the major component of normal gamma globulin, appears in the first column effluents. Those gamma globulins with higher molecular weights (sedimentation coefficients greater than 7S) have appeared in subsequent effluents. The 19S gamma globulins (molecular weight, 900,000) have been eluted at the tail end of the chromatogram.

This type of chromatography in combination with paper electrophoresis of the eluted fractions has made it possible to quantitate the amounts of gamma globulins of differing molecular weight in serum and in this way to distinguish normal, hyperglobulinemic and macroglobulinemic sera

from each other. Especially useful has been the capacity of the technique to distinguish between the serum of multiple myeloma and Waldenstrom's macroglobulinemia. The fractions obtained by chromatography have, in all cases, been characterized in the analytical ultracentrifuge.

Sera from normal individuals and from patients with a variety of hyperglobulinemias including those of Waldenstrom's macroglobulinemia, multiple myeloma, hyperglobulinemic purpura, liver disease, agammaglobulinemia and rheumatoid arthritis have been fractionated. Diagnostic information comparable to that available from the analytical ultracentifuge has been obtained. The ultracentrifugal data will also be presented.

The chromatographic technique has also offered a means of preparing normal and macro gamma globulins. It has been possible in this way to isolate and characterize the rheumatoid factor as a 19S gamma globulin and the gamma globulin of hypogammaglobulinemia as a mixture of light and heavy gamma globulin. Finally, it has been possible to prepare pure 7S gamma globulin for immunological purposes by a single elution step. These procedures will also be described.

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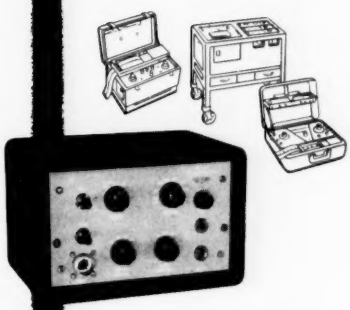

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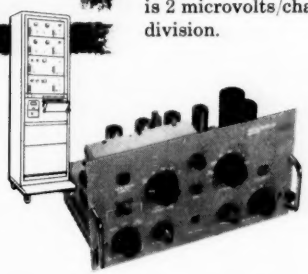

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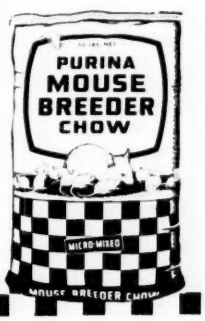

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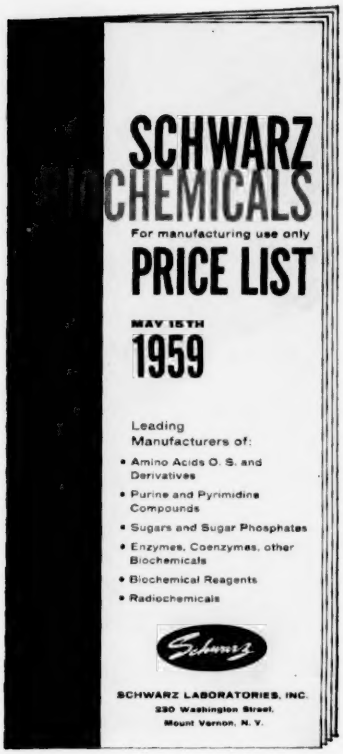

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